

**Aspects of the Biology of *Papaver*
bracteatum Lind, a New Crop for Tasmania**

By

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**Submitted in fulfilment of the requirements for the Degree of
Doctor of Philosophy**

University of Tasmania

Hobart

March 2011

DECLARATION

I hereby declare that this thesis does not contain any material which has been accepted for a degree or diploma by the University of Tasmania or any other institution. To the best of my knowledge, this thesis contains no material previously published or written by another person except where due reference is made in the text of this thesis.

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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Prof. Philip Brown for his guidance, constant oasis of ideas and passion for science, which has inspired and enriched my development as a student, and scientist. I am indebted to him more than he knows.

I gratefully acknowledge Dr. Alistair Gracie for his advice, supervision, and crucial contribution, which made him a backbone of this research and so to this thesis. His involvement with his originality has triggered and nourished my intellectual maturity that I will benefit from for a long time to come. His never say “no” attitude for any silly and painful questions I have asked him over the past two years makes him one of the ‘coolest’ persons I have ever met.

I gratefully acknowledge the support of Phil Andrews (Manager, Horticulture Research centre) in setting up glasshouse trials. His technical knowledge is second to none. Dr. David Ratkowsky and Dr. Samuel Adediran provided expert statistical advice. Andy Measham and Angela Richardson provided laboratory support, and Sally Jones and Jane Bailey provided administrative support. Thank you!

I would like to acknowledge Dr. Karsten Goemann for assistance with operating the ESEM and Dr. Cameron Spurr for allowing me to use his seed grading equipment. I would also like to thank Dr. Peter Lane and Dr. Richard Doyle who have been helpful at various stages of my candidature.

The entire journey of 4 years of research would not be possible without the support of my friends Jane Evans, Anchana Ganesan, Meenatshi Nachiappan, Una Evans, Naveen Duppati, Dr. Bhim Khatri, Dr. Samuel Adediran, Dr. Shaun Sutor, Kieren Rix, Dr. Ifayanti Ridwan Saleh, Dr. Mandeep Kaur, Ang Geard, Penny Measham, Jessica Howard, Marek Matuszek, Tharshini Thiru, Wahyuni Husin, Anne Koshy, Dr. Lyndal Mellefont, Steven Ridge, Marcus Hardie, Sriram Padmanabhan, and Rolf Nilsson. To all my facebook friends, thanks! I apologise, “Mate”, if I inadvertently missed you off this list.

It has been an honour to meet Prof Arielevy, who has worked with *P. bracteatum* for the past twenty years. Many thanks to Prof. Levy for supplying the seeds for this project. I have had the privilege of meeting Dr. John Laughlin. His enthusiasm for and knowledge of poppies is truly amazing.

Finally I would like to dedicate my thesis to the special people in my life who believed in me and made my long term dream of being a Doctor a possibility.

Prof. Phil Brown (Mentor)

Dr. Subba Rao Madam (Dad)

Mrs. Vara Lakshmi Madam (Mom)

Aravind Madam (M.Tech) (Brother)

ABSTRACT

Papaver bracteatum L, a species that accumulates high concentrations of the pharmacologically important alkaloid thebaine in its fruit capsules, has been identified as a potential new perennial crop in Tasmania, Australia. Previous attempts to establish the crop from seed in Tasmania have met with limited success, with slow and irregular emergence reported under field conditions and low flowering percentages in the year of planting. The recent availability of improved germplasm and advances in agronomic techniques associated with crop establishment has provided the impetus for a re-evaluation of *P. bracteatum* as a new horticultural crop. This project focussed on understanding aspects of the biology of *P. bracteatum* needed for the development of production strategies for the successful commercialisation of this crop in Tasmania, Australia.

Papaver bracteatum seeds displayed no physiological dormancy and germinated in both light and dark conditions. The optimum temperature range for the germination was found to be between 18° and 25°C, with lower and upper cardinal temperatures of 5°C and 35°C respectively. During storage, a decrease in germination viability of between 0.2% and 0.1% per week was recorded over a period of 100 weeks under 20°C and ambient humidity conditions. Germination was found to be sensitive to water stress, with germination inhibited at water potentials of -0.1 and -0.3 MPa. It was concluded that sensitivity to water deficits may explain poor field emergence. Grading of seed-lots to retain the larger sized and denser seed fraction improved

germination percentage and seedling vigour. During seed production, harvesting after the 70 DAFB resulted in the highest seed quality.

Seedlings required an extended period of juvenile growth before they became competent to flower. Plants were capable of initiating flowering 20 weeks after planting when grown under glasshouse conditions and approximately 30 weeks after planting when grown under shade house conditions. The transition from juvenile to mature stages was found to correspond to a fully expanded leaf number of approximately 17. Leaf morphological characteristics used as phase change indicators in other species were shown not to be applicable in *P. bracteatum*. Trichome distribution and density, leaf size and plant height, which was a measure of leaf erectness and leaf length, varied considerably with growing conditions and did not provide a consistent value at the phase change that could be used as an indicator.

Papaver bracteatum was shown to require a period of vernalisation to induce flowering. Results suggested that less than 4 weeks exposure to vernalising night temperatures can induce flowering that and adequate vernalisation may occur within a night temperature band of at least 5° to 10°C. Observations also led to the conclusion that, unlike the annual opium poppy species *P. somniferum*, *P. bracteatum* does not have a daylength sensitive flowering response. Application of gibberellic acid to plants grown under marginally inductive conditions induced more rapid and more even flowering within the plant population. Knowledge of the flowering response in *P. bracteatum* was used to develop recommendations for planting time and production environment to ensure flowering and therefore high capsule yields in the season that the crop is planted.

GLOSSARY

ANOVA	Analysis of variance
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAI	Days after imbibition
df	degrees of freedom
DAFB	Days after Full Bloom
DPIWE	Tasmanian State Government Department of Primary Industries and Water and Environment
DPIPWE	Tasmanian State Government Department of Primary Industries, Parks, Water and Environment
ESEM	Environmental Scanning Electron Microscope
GA	Gibberellic acid
GSK	GlaxoSmithKline
HRC	Horticultural Research Centre
INCB	International Narcotics Control Board
IPM	Integrated Pest Management
ISTA	International Seed Testing Association
LDP	Long day plant(s)
LSD	Least Significant Difference
P	Probability
PACB	Poppy Advisory Control Board
PEG	Polyethylene Glycol
SAS	Statistical Analysis Software
SD	Short day(s)

SEM	Standard Error of the Mean
STDEV	Standard deviation
UN	United Nations
USDA	United States Department of Agriculture
USSR	Union of Soviet Socialist Republics
WHO	World Health Organisation

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PUBLICATIONS

CONFERENCE PRESENTATION

Madam, PRK and Brown, PH (2007), Introduction of a Potential New Crop for Tasmania: Sharing Excellence in Research, Tasmanian University Postgraduate Association (September, 2007). First prize in the Sustainable Production Category for the Best Presentation.

CONFERENCE POSTER

Madam, PRK, Brown, PH and Gracie, AJ (2008), *Seed dormancy, germination and plant juvenility studies in Papaver bracteatum*, Proceedings of the National and Trans Tasman Horticultural Science Conference, 21-23 July 2008, Gold Coast, Queensland [Conference Extract]

CHAPTER 1

PAPAYER BRACTEATUM: A POTENTIAL NEW CROP FOR TASMANIA

The genus *Papaver* contains several species that are unique in their ability to synthesize a group of chemicals referred to as the opiate alkaloids. These opiate alkaloids are heavily utilized in the global pharmaceutical industry as well as being a significant problem as part of the illicit drug industry. At present, one species in the genera, *Papaver somniferum*, is the source of both licit and illicit opiate alkaloid supply. Commercial cultivation of *P. somniferum* has occurred in Australia since 1970's for medicinal and pharmaceutical industries. In Australia, the state of Tasmania is the commercial poppy growing region and currently produces approximately 40% of the world's legally traded opiates (Chitty *et al.*, 2003). Estimates are that the world market demand will see a continued expansion at the rate of at least 5% per annum in the near future (DPIWE, 2004).

Tasmania has been assessed as having suitable land area to expand production by up to three fold and Tasmanian standards in all aspects of poppy production are considered to be amongst the best in the world (Fist, 2001). Fertile soils, a maritime temperate climate, and the availability of irrigation to supplement natural rainfall make Tasmania an ideal location for growing poppies. Although, the poppy industry has made significant progress in its relatively short history in Tasmania, there is still scope for improvements in research and development to ensure that the poppy industry maintains its high standard of efficiency and productivity and also by

introducing new commercial varieties of poppy which are highly valuable for mankind (DPIWE, 2004). One area of research open to the Tasmanian industry is the investigation of alternative *Papaver* species as sources of opiate alkaloids to complement *Papaver somniferum* production. *P. bracteatum* has been identified as a species with potential as a new opiate producing crop in Tasmania, Australia. The Opium poppy (*Papaver somniferum*) is the global commercial source of medicinal opiates and related compounds. Morphine, codeine, thebaine, and narcotine are the most important alkaloids produced by *P. somniferum*. Most of these compounds are widely used in the pharmaceutical industry as analgesics and anti-spasmodics, and demand for the opiates has been rising (Schmeller and Wink, 1998; Tetenyi, 1997). The increasing world-wide legitimate demand for codeine must be met by either the increased production of alkaloids from *P. somniferum*, with the associated increased risk of its diversion into illicit channels, or identification of alternative sources of opiates with lower risk of illicit use. The cultivation of *P. bracteatum* as an alternative opiate alkaloid source is one such strategy to improve licit opiate supply without the risk of diversion to illicit uses.

P. bracteatum produces the opiate thebaine, a medicinally important alkaloid, and is characterized by complete absence of morphine synthesis (Nyman, 1979). Neubauer and Mothes (1963) reported that some plants of *P. bracteatum* contain 98 percent thebaine of the total alkaloids present in different parts of the plant. Domestication of this wild species has come into consideration because of the abuse of derivatives of opium and heroin which are the main products of opium poppy (*P. somniferum*). Illegal operations involved in manufacturing these drugs, and the rise of crime and corruption in society associated with the illegal drug trade, have fuelled the

development of international programs to find an alternative crop. The thebaine produced by *P. bracteatum* requires a complex process to be converted to codeine, the initial material used in illicit opiate drug production, hence making its illegal use very difficult (Lalezari *et al.*, 1974). In addition, *P. bracteatum* has been found to be very high yielding. Sharghi and Lalezari (1967) found that 26 percent of the dry latex from the plant was opiate alkaloids and this represented 3.5 percent alkaloid yield in the dry, ripe capsules.

A further advantage of *P. bracteatum* is that, unlike *P. somniferum*, it is a perennial species. By incorporating perennials into traditional cropping systems, a wide range of benefits can be gained. Perennial crop species remain productive for many years hence reducing the need for re-establishment which is expensive in terms of labour and resources. These crops generally require less water for their growth and also reduce the risk of dry land salinity. Perennials also reduce soil erosion by providing a year long ground cover, increased soil porosity and nutrient content, and a decrease in tilling and seeding (Lefroy *et al.*, 1999; Scheinost *et al.*, 2001). Farming systems incorporating the use of perennial crops may, therefore, provide environmental and economical benefits to annual cropping systems.

In addition to potential use as an alkaloid source, a secondary, but important purpose of *P. bracteatum* cultivation may be for use in the food industry to produce poppy seed and poppy seed oil (Duke, 1973). The seeds contain 45-48% oil, rich in unsaturated fatty acids. Calculated mean seed yield and seed-oil yield have been about 90 kg/ha and 40 kg/ha, respectively (Seddigh *et al.*, 1982). Whole seeds of *P. bracteatum* are used in the confectionery and baking industries in western Iran (Balbi,

1960). The amount of seed-oil produced by this species depends on the age of the plant and the time of capsule harvest. The yield is notably lower than yields reported for opium poppy. However, seed-oil quality is high and can be used for cooking and industrial purposes (U.N.Secretariat, 1974).

There are two further aspects of some importance concerning *P. bracteatum* which supports its introduction to Tasmania as a new crop. A number of cultivars have been developed and produced as ornamental plants (Nemeth, 1998), highlighting the attractive appearance of the species when in flower. In November and December, fields of pink *P. somniferum* poppies have already become a tourist attraction in different areas of the island state, and cultivation of the new species with more attractive flowers may be expected to add to this appeal. Poppies have also gained importance in the honey bee industry in Tasmania. Honey bees have been previously identified as a major pollinator of poppies in Europe (Tetenyi, 1997) as well as being frequent visitors to poppy crops in Tasmania (Miller *et al.*, 2005). Increased production of the various industries dependent on honey bee pollination was identified by a recent Honeybee Industry Linkages Workshop in Tasmania as critical to maintaining viability of the honey industry (DPIWE, 2004). The key benefits in integrating management of pollination services with honey production are maximizing returns for beekeepers and ensuring greater productivity for farmers through increased crop yields, improved quality and reduced flowering time. Therefore, in a similar way to *P. somniferum*, the cross pollinated *P. bracteatum* with even more visually attractive, dark blood-red petals could also be very advantageous to the tourism and honey industries in the state.

AN ALTERNATIVE SOURCE OF MORPHINE

Papaver somniferum, the opium poppy, is one of the most famous, and infamous, plants used by man. No other plant species relieves and causes so much suffering. Pharmaceutical derivatives, morphine and codeine, which are refined from opium poppy and administered under government license, are powerful pain relievers and are a critical part of many modern medical procedures. In contrast, illicit derivatives from traditional poppy such as opium and heroin have a negative effect on the lives of addicts (Neild, 1987). Usage of these illegal drugs in different areas and countries is governed by the factors such as availability, price and social acceptance. The use of opium, by smoking and by oral ingestion, has a history of medical and social acceptance in many parts of the world, not only in the opium producing countries of Asia but also, during the 19th and very early 20th centuries in Europe and North America (Brian, 1994; Jonnes, 1995) where it was regarded as a serious form of addiction.

The most widely used opiate, codeine, is derived from morphine which is isolated from opium, the dried latex extracted by lancing of green poppy capsules, or from poppy straw, the dried poppy capsule and stem material harvested from senesced *P. somniferum* plants. Unfortunately, morphine may be misused by converting into its diacetyl-derivatives (heroin). To address this problem at the source level, the search for an efficient alternative synthetic or natural drug to replace opiates or for a source of codeine other than from the well established opium poppy, has commenced to assist in reducing the illegal market of heroin.

Predictions that synthetic drugs would replace the natural narcotic drugs (Beyerman *et al.*, 1976) have not been substantiated, and in the 1980's the United Nations concluded that synthetic narcotic drugs had not gained any significant share of the market. Therefore, greater attention has been given to finding a new natural source of morphinan alkaloids as an alternative to opium poppy. This search focused attention on *P. bracteatum* which proved the most promising out of all the *Papaver* species. Thebaine extracted from *P. bracteatum* can serve as an excellent substitute for morphine as a starting material for its chemical conversion into codeine by a process called as demethylation (Mallinckrodt, 1974). The most widely used opiate codeine has been recognized as an effective pain reliever and cough suppressant. The chemical conversion of thebaine into heroin requires both advanced chemical skill and equipment, thereby making such a procedure less likely to be used illicitly.

Thebaine is an important source material for the production of anti addiction drugs such as naloxone, buprenorphine, oxycodone and hydrocodone (McNicholas and Martin, 1984) as well as for codeine. Codeine is also used as a mild analgesic prescribed for the relief of cancer, and head trauma. Naloxone is used as an antagonist in the treatment of morphine and heroin addicts. Etorphine and other compounds derived from thebaine have a much greater activity than morphine and are used in veterinary medicine for sedating wild animals (Bentley, 1971). Thebaine utilization has increased from 5–8 MTs per year between 1981 and 1994 to 45.6 MTs in 2000 (Anon, 2002; Shukla *et al.*, 2006).

In recent years the demand for opiate raw materials rich in thebaine has increased globally. The total demand for opiate raw materials rich in thebaine was estimated

about 140 tonnes in 2008 and 160 tonnes in 2009 respectively. Increased demand for thebaine-based opiates, which was concentrated mainly in the United States, has increased sharply because of the spread of usage of these opiates to other countries (INCB, 2009). Few studies have reported on the addiction liability of thebaine, its derivatives and minor alkaloids although no case of thebaine abuse or illicit productions of its derivatives have been reported (Theuns *et al.*, 1986). Thebaine represents a very large percentage of the total opiate alkaloid yield from *P. bracteatum*. A total of 27 alkaloids have been found, with alpinigenine and isothebaine being the most prevalent after thebaine and with only trace amounts of other alkaloids including alpinigenine, orientalidine and isothebaine (Nyman, 1979), oripavine (Kiselev and Konovalova, 1948), salutaridine (Heydenreich and Pfeifer, 1966), codeine, neopine (Kuppers *et al.*, 1976), and protopine (Bohm, 1974) reported. The minor alkaloids of *P. bracteatum* occur in very small amounts and can be easily isolated from other plant species (Kettenes-Van den Bosch *et al.*, 1979) and thus, the abuse potential of alkaloids from *P. bracteatum* is negligible.

POLITICAL AND CULTURAL CONSIDERATIONS

Despite the identification as early as 1967 of *Papaver bracteatum* as a potential alkaloid producing crop by Sharghi and Lalezari (1967), at present the species is not grown commercially anywhere in the world. The commercial development of *P. bracteatum* has been delayed as much by political and cultural considerations as by agronomic problems (Seddigh *et al.*, 1982). The main political problem is that many small scale growers of *P. somniferum* in the traditional opiate producing countries of India and Turkey would suffer severely if *P. bractetaum* grown in other countries

became the preferred source of opiates. For this reason UN Resolution 471, largely, restricts the export of opiate raw material to these two countries (Theuns *et al.*, 1986).

On the assumption that pressure to allow the widespread cultivation of *P. bracteatum* will ultimately be successful, it has been argued that a coordinated effort should be made to develop effective crop substitution programs in India and Turkey (Cochin, 1975). This approach would be particularly important in India where poppy alkaloids are extracted from labour intensive opium production, contributing significantly to rural employment, rather than from poppy straw as in Turkey. In addition the possibility of a shift to *P. bracteatum* cultivation is far less likely in India than in Turkey because of climatic restriction in the current *P. somniferum* production regions (Krikorian and Ledbetter, 1975).

A number of aspects of *P. bracteatum* biology have also been raised as possible barriers to its establishment as a crop. The species requires cross-pollination and previous attempts to develop self-fertile lines of *P. bracteatum* were unsuccessful, potentially raising regulatory issues associated with the production of seed required for crop establishment (Bohm, 1981). Seed shattering may also be a problem as the capsules of *P. bracteatum* develop dehiscent pores under the stigmatic disc at maturity, and seed losses from the capsules may create problems in controlling the spread of the plants (Seddigh *et al.*, 1982). The potential for breeding or selection of plants with indehiscent capsules (Levy *et al.*, 1986) provides a mechanism to overcome this issue.

While political and cultural problems may be barriers to widespread *P. bracteatum* cultivation, other issues are likely to be more significant barriers to production in Tasmania. As with the introduction of any new crop, questions of climatic suitability, agronomic practice and economic potential require answers in the evaluation of the species as a new crop. The species is at present poorly characterized in terms of its agricultural potential.

HISTORY AND ORIGIN

P. bracteatum grows wild in the Alborz Mountains in the north and northwest of Iran on the north slopes facing the Caspian Sea (Goldblatt, 1974), the Kurdistan region of western Iran and on north-facing slopes of the Caucasus Mountains in the USSR (Seddigh *et al.*, 1982). The species is well adapted to relatively dry conditions and usually grows on stony slopes from 1,500-3,000m above sea level (Goldblatt, 1974; U.N.Secretariat, 1973). *P. bracteatum* is one of the three species belonging to the section Oxytona of family Papaveraceae. The species in section Oxytona are very much alike, and incorrect botanical identifications resulted in much confusion in the literature with their alkaloid contents and chromosome numbers (Tetenyi and Zambo, 1978). All species in section Oxytona are perennial and are easily distinguished from other Papaver species. A complete literature review of the taxonomy of this species has been carried out by Goldblatt (1974).

Plants of these species were first brought to Europe early in the eighteenth century by Tournefort and were introduced as „oriental poppy’ (Goldblatt, 1974). Research has shown that *P. bracteatum* is well adapted to growing conditions in Europe, North America, Israel, and India (Fairbairn and Hakim, 1973). The earliest collection of *P.*

bracteatum was given to the United States Plant Introduction Station (USDA, Beltsville, MD) by I. Lalezari of the University of Tehran who recognized the social and economic potential of the species (U.N.Secretariat, 1974) and the collection was registered in 1972 as PI 368264. Several additional collections from various regions of Iran have since been introduced and registered in the USDA Plant Introduction Station. In 1975, Oregon State University received several seed collections from the USDA Western Regional Plant Introduction Station at Pullman, WA, and began investigating its potential as a commercial crop. According to Fairbarn and Helliwell (1977) the plants remain economically productive for a period of 8-10 years and some plants were viable up to 15 years (Mallinckrodt, 1974). This perennial nature makes it an attractive option to introduce *P. bracteatum* as a commercial crop in Tasmania.

BOTANICAL DESCRIPTION

Papaver bracteatum was first recognised as a species by Lindley in 1821 (Coffman *et al.*, 1975) and is closely related to *P. orientale* L. and *P. pseudo-orientale* (Goldblatt, 1974). A recent bio-systematic study of these 3 species which belong to section Oxytona of Papaveraceae has provided a framework for distinguishing the species based on morphological, cytological, and alkaloid chemical criteria. All species of section Oxytona are perennials and are characterized by their rosette growth habit. The leaves are simple or often dissected, and arranged to form rosettes. The basal part of the stem may be horizontal with a hairy or glabrous epidermis. The floral axis has no lateral branches or leaves and is glabrous or covered by erect white or brownish black hairs bearing single flowers. The two oval sepals usually fall off after flower opening, with the exception of some arctic species where they remain for

a longer period after flowering. The petals are wedge shaped, growing laterally and more or less overlapping (Goldblatt, 1974) and usually fall after flowering, but in some cases can remain attached to the surface of the ovary. Flowers are highly variable in colour, sometimes with basal marks of different colours.

The herbaceous perennial poppy derives its botanical name *P. bracteatum* from the presence of typical bracts that are retained after flowering, under the capsules. The flowers have four to six deep red coloured petals with black stripes and are characterized by a rosette arrangement of leaves. The leaves are hypo-stomatic, deeply incised multi-serrate hairs on the adaxial epidermis. The flower buds of *P. bracteatum* are erect throughout their growth with broadly spread calyx bristles. The fruit is a dry capsule about 3cm long and 2g in weight, covered with a large flat stigmatic disc. During maturation, the stigmatic disc separates from the capsule, thus enabling scattering of the seed through pores of dehiscence (Goldblatt, 1974). After maturation of the capsules, the aerial parts dry out and the plant remains dormant during summer until a new vegetative growth resumes in the following winter. The pigments of the petal do not fade on storage and they contain pelargonidin-3-glucoside which is the key to distinguish the species from others by using paper chromatography (Fairbairn and Hakim, 1973). Roots of *P. bracteatum* are long, unbranched and contain a high amount of alkaloid. It is an out-crossing species with the prominent flowers of the plant attracting several insects including bees and beetles that are involved in pollination (Goldblatt, 1974).

In young plants of *P. bracteatum*, thebaine content increases rapidly in roots followed by an increase in concentration in the shoots after a few weeks. During its

second year of growth the highest concentration of thebaine is found in the capsule four to six weeks after flowering. If budding is prevented, the thebaine content remains high in the roots (Cordell, 1981). Alkaloid biosynthesis in *P. bracteatum* is not limited to any part of the plant (Mothes *et al.*, 1985), unlike *P. somniferum* where alkaloid accumulation is essentially restricted to the capsule.

AUSTRALIAN POPPY INDUSTRY

Poppies (*Papaver somniferum* L.) were grown in Australia on a very small scale throughout the 19th century by some medical practitioners for the production of opium to be used in their individual practices (Walker, 1977). This practice had ceased by the early 20th century with supplies of medical morphine being available for European exporters (Bernath and Tetenyi, 1982). Poppy production in Australia did not commence at a commercial scale until after World War Two, with supplies of medicinal morphine being imported from Northern Hemisphere countries to meet domestic demand. However in the early 1960s an experimental program of poppy production was commenced in Tasmania by the English pharmaceutical company McFarlane Smith, a subsidiary of Glaxo. The motivation for this resurgence of interest was that the major pharmaceutical companies in the Northern Hemisphere drew their supplies mainly from India and Turkey with small amounts from Eastern Europe. Supplies of poppy straw and opium from these traditional areas of production were subject to fluctuation because of the vagaries of weather and production problems. A strategy was developed to draw supplies of morphine and related alkaloids derived from dry poppy straw from Australia. In addition, Australia is politically stable with modern agricultural expertise, infrastructure and reliable climatic conditions. Reliable supply of poppy alkaloids, and the out-of-season nature

of production in the Southern Hemisphere, spread the supply of poppy straw and derived pharmaceuticals to complement supplies drawn from the Northern Hemisphere (Laughlin *et al.*, 1998).

By the late 1960s, commercial production of poppies had begun in Tasmania with farmers being contracted by Glaxo Australia Pty Ltd (now GlaxoSmithKline) to grow the crop. In the early 1970's a second pharmaceutical company, Abott International, entered the industry by the name of Tasmanian Alkaloids. It is now owned by the large American pharmaceutical company Johnson and Johnson. A formal agreement between all of the six Australian states was made in 1971, and the production of poppies was exclusively restricted to Tasmania. The main reason for this decision was the isolation of Tasmania, an island state of Australia, from the main land which gave added security against any illegal movement of poppy crop across state borders. Since then the poppy industry has expanded in Tasmania to become one of the states most important agricultural industries, with production valued at over A\$400 million in 2008 (DPIPWE, 2009).

Poppy production on private farms in Tasmania is administered by a system of licenses issued under the direction of the State controlled Poppy Advisory and Control Board (PACB). Licenses are only issued after farmers have met a stringent range of criteria. Inspectors from the PACB monitor all crops throughout the growing season for any evidence of illegal use and to ensure that crop straw is properly disposed of after harvest to minimize any re-growth problems. In the 2008-2009 seasons about 25,000 hectares of poppies were cultivated in Tasmania and the total area which has been used since 1970 has ranged from about 500 to 25,000 ha

(Fist, 2001). The stringent security measures along with the isolation of Tasmania have ensured that any illegal use of crops has been minimal.

POPPY PRODUCTION IN TASMANIA

Poppies are grown in rotation with vegetable crops in the northwest, and cereal crops in the midlands and south, and the farming systems present in the state have proven Tasmania to be an excellent location for the poppy industry (Fist, 2001). Only one major perennial crop is incorporated in the vegetable and cereal cropping systems. Pyrethrum, an herbaceous perennial which produces a natural insecticide, was introduced in Tasmania as early as 1980's and has been widely adopted by the farmers as an important plant in the crop rotation system. Production of Pyrethrum was considered to be more profitable than pastures in the crop rotation with the existing annual poppy (*P. somniferum*). The benefits of having a perennial crop in an annual vegetable and poppy rotation have been documented and include improvement of soil physical condition, reduction of soil erosion, decreased irrigation requirements and most importantly reduced farm capital investments (DPIWE, 2006). Increasing focus on soil health and the limited availability of irrigation water has stimulated interest in other perennial crops that may be incorporated into the cropping systems.

Poppy production in Tasmania is carried out in a cool temperate environment. Large areas of crop are produced in the North West region of the state and cultivated between latitude 41°S to 42° 30' S (Laughlin *et al.*, 1998). Tasmania has a mean maximum temperature of 21°C (70°F) in summer (December to February) and 13°C (55°F) in winter (June to August). Tasmania enjoys a temperate, maritime climate

with four distinctly different seasons. Tasmania's summer days are long with daylight lasting more than 15 hours and darkness not falling until 9.30 pm daylight saving time in mid-summer. This cool temperate climate allows the plant to grow well during summer and the dry summers facilitate harvesting of crops with little need of artificial drying. Geographical advantage combined with technological developments and skilled farmers have enabled Tasmania to achieve the world class status as a leader in poppy production.

The poppy ecotypes cultivated in Tasmania are long-day plants that are specially developed for Tasmanian conditions and have been bred for the basic fundamentals of high capsule yield and high concentration of poppy alkaloids, especially morphine and codeine. Apart from alkaloid content, the other factors that are incorporated in to the various breeding lines include straw length, standing ability and disease resistance (Laughlin *et al.*, 1998).

A number of cultivars with different alkaloid profiles have been bred and are grown in Tasmania, with cultivars producing predominantly thebaine becoming increasingly important to the industry. A spontaneously occurring mutant with increased production of thebaine and low content of morphine was observed in *P. somniferum* by (Nyman and Hall, 1976). Since then the repeated selection process led to the present day thebaine producing varieties in *P. somniferum*. In Tasmania, the alkaloid content in the harvested poppy straw is generally in the range of 1.5% to 2.7% on a dry weight basis. The higher the alkaloid content the higher will be the financial return to the growers and in a competitive market (Chitty *et al.*, 2003). Alkaloid content has doubled in Tasmanian poppy industry as a result of conventional plant

breeding and has kept track with increases in other parts of the world generated through breeding and genetic transformations in the last two decades (Facchini *et al.*, 2007).

The germplasm used and the seed selections developed in Tasmania have been the exclusive and independent prerogative of the two contracting pharmaceutical companies, GlaxoSmithKline and Tasmanian Alkaloids. The combination of germplasm and seed selection, climatic environment, soil type and farming techniques have resulted in the alkaloid yields per hectare of Tasmanian poppies being the highest in the world (Laughlin *et al.*, 1998). In Tasmania, the preferred sowing time for most poppy crops is early spring, with flowers produced in December and a dry mature harvest (12% moisture in capsules) during February/March. Autumn sowing in the drier southern areas of the state, with mean annual rainfalls of 500-600 mm compared to 900-1000mm in the North West region, has generally shown lower capsule yields with lower alkaloid levels. Currently all poppy crops in Tasmania are spring sown (Laughlin *et al.*, 1998).

PAPAYER BRACTEATUM IN TASMANIA

Papaver bracteatum was first examined in Tasmania in the 1980's as a potential new crop. The species is endemic to mountainous regions with cool climates, and was considered likely to grow well in cooler climatic conditions such as those found in Tasmania (Laughlin *et al.*, 1998). However all previous efforts to establish *P. bracteatum* as a commercial crop in Tasmania were unsuccessful. Previous studies indicated that *P. bracteatum* was not competitive commercially with the annual *P. somniferum* because of delayed flowering and low first season yield due to its perennial growth habit, and difficulty in crop establishment in the first year (Fairbarn,

1976). Advances in crop agronomic practice in Tasmania over the past 20 years, and in particular improvements in irrigation technology and availability of pre-emergent herbicides, may assist in overcoming the crop establishment difficulties noted in the earlier trials. The availability of new genetic material, selected from the plant evaluation program of Prof Arie Levy in Israel (Levy, 1985), may also address the problem of poor first year flowering rates. These improvements in research prompted a re-evaluation of the potential of *P. bracteatum* as a potential new crop for Tasmania by a newly established commercial alkaloid production company in the state, TPI Enterprises Pty Ltd.

RESEARCH ON *P. BRACTEATUM* CROP PRODUCTION

The Fourth Working Group of the United Nations Division of Narcotic Drugs reviewed the research on *P. bracteatum* and recommended that maximizing the harvested yield of this species would ensure that world demand for codeine was satisfied and would avoid over-production of illegal alkaloids. Despite this recommendation, little work appears to have been done and no research on agronomic approaches to increasing yield has been published in the publicly available literature. The few references to *P. bracteatum* in the literature are limited to its taxonomy and alkaloids, and information on its life cycle and environmental requirements is unknown. Aspects of plant biology critical to crop production, including seed dormancy, germination characteristics and flower initiation and development processes, have not been documented.

The seeds of *Papaver bracteatum* are very small and contain fatty oils (palmitic acid 7.5%, stearic acid 2.5%, oleic acid 10.0%, linolic acid 78% and traces of 0.6%

arachidic acid) similar to composition present in *P. somniferum* (Nyman, 1979). No dormancy requirement has been documented. Seeds of *P. somniferum* are known to be capable of germination soon after being shed from the capsule (Acock *et al.*, 1997), and ornamental, perennial species of *Papaver* are known to readily establish following self seeding in a garden situation (Bohm, 1974), suggesting that few if any dormancy mechanisms are present and that germination may proceed under variable environmental conditions.

The small size of *P. bracteatum* seed may restrict longevity in storage after harvest, as has been noted in general for small seeded species (Duke, 1973) but no data on *P. bracteatum* seed storage has been published to confirm this assumption. In addition, the small size of the seed suggests that shallow sowing may be required and that like other shallow sown crops irrigation during crop establishment may be critical to achieving rapid and uniform crop emergence. Characterization of germination responses of *P. bracteatum* seed under varying water potential and temperature conditions has not been previously documented. Bare *et al.* (1978) studied the seed germination characteristics of three *Papaver* species; *P. bracteatum*, *P. orientale* and *P. somniferum*, under different light and temperature regimes. Optimal temperatures range from 18° to 30°C in *P. orientale* and 13° to 33°C in *P. somniferum*. An optimum temperature of for germination of 21° to 26°C was proposed for *P. bracteatum*, but upper and lower thresholds were not determined.

As with seed germination responses, little is known about flower initiation in *P. bracteatum*. Low rates of first season flowering following autumn sowing in the early trials in Tasmania suggests that an extended juvenility phase may be present in

the species, but this has not been investigated or characterized. Based on its centre of origin and documented spring time flowering pattern in its natural habitat (Levy and Palevitch, 1982), a vernalisation requirement for flower initiation may be expected if any environmental requirements for flowering are present in the species. The annual species *P. somniferum* displays a day length sensitive flowering response (Wang *et al.*, 1996), and a similar requirement in *P. bracteatum* cannot be ruled out. Mika (1955) reported that *P. somniferum* plants flower when grown initially in non-inductive conditions and transferred to inductive conditions within a short period. These results were later confirmed by Acock *et al.* (1996).

While little research on the biology and agronomy of *Papaver bracteatum* appears to have been undertaken over the past 20 years, advances have been made in plant selection and in breeding projects to develop varieties better adapted to environmental conditions. Significant advances have been made in breeding high thebaine producing varieties by a research team led by Prof. Arie Levy who studied selection and domestication procedures. A spontaneous mutant of *P. bracteatum* with closed capsules was identified by Levy (1985) and minimized the loss in seed yield. The shattering resistant mutant identified has no pores of dehiscence and could easily be recognized by the size of the stigmatic disc. Also, the mutant capsules have the ability to produce higher thebaine content than the normal capsules. Closed capsules and high yield potential are attributes that may be combined with other growth and development traits to deliver cultivars suited to Tasmanian production conditions.

CONCLUSIONS

The introduction of *P. bracteatum* into the Tasmanian poppy industry as a new crop may deliver the industry a number of potential advantages based on its perennial nature, high thebaine yield, attractive appearance at flowering and attractiveness to bees. The economic viability of the crop will however be dependent on achieving high capsule yields, particularly in the first season when the costs of crop establishment must be defrayed, in addition to high thebaine content. The high cost of labour in Tasmania reduces the viability of vegetative crop establishment methods, so establishment of an even plant stand following autumn seed sowing is required if high flower yields are to be obtained in the first season. In addition, provision of suitable growing conditions to promote flower initiation and development will need to be met through site selection, crop management practices and cultivar selection. The knowledge of *P. bracteatum* biology needed to ensure producers are able to meet these requirements is currently not available.

One of the first steps for introducing a new species into cultivation is to understand the environmental control of its phenology. Aspects of seed biology, length of the juvenile phase and environmental control of flower initiation and development in *P. bracteatum* were identified in the review of literature as key areas where research is required. This project therefore sought to provide knowledge of these aspects of *P. bracteatum* biology to assist the poppy industry in Tasmania to establish the species as a new crop.

CHAPTER 2

GENERAL MATERIALS AND METHODS

PROJECT OVERVIEW

This project commenced in March, 2006 and experimental work was completed in January 2010. The research undertaken in the project characterised aspects of seed germination, juvenility and floral biology of *Papaver bracteatum*. Due to difficulties in maintaining healthy plant growth under glasshouse conditions encountered during the project, greater emphasis was placed on seed biology studies. Experimental work conducted throughout the project has been divided into four areas (germination responses, seed quality, juvenility and flowering), and each area is presented as a separate research chapter. A number of materials and methods were common to two or more chapters and are therefore presented in this chapter to avoid duplication.

PLANT MATERIALS

Papaver bracteatum seeds were sourced from Prof. Arie Levy (Volcani Institute, Israel), and all experimental work was undertaken using material from a single cultivar or selected line from Prof Levy's plant improvement program. As *P. bracteatum* is a prescribed species in Tasmania due to its narcotic alkaloid content, all experimental work was undertaken under permit from the Poppy Control Advisory Board in Tasmania. Imported seed material was stored under refrigerated conditions in secured premises until required for experimental work. Each year, new

seed stock was generated from healthy plants grown under shadehouse conditions at the University of Tasmania. Plants were hand pollinated and seed harvested from individual capsules was labelled and stored in separate bags to enable experiments to be conducted on single capsule seed lots or, where larger numbers of seeds or plant were required, from mixed seed lots. Prior to seedling production for juvenility and flowering experiments, seed lots were graded using size and density separation to remove impurities and the very light seed fraction. Also, one of the key challenges undertaken during the course of study was lack of sufficient plant material and genetic diversity of population.

Seed germination experiments were conducted at the Sandy Bay campus of the University of Tasmania, Hobart (S 42° 54.306', E 147° 19.467' at an elevation of 55m). After drying procedures, mature seeds extracted from the capsules were sieved using a vertical air column (South Dakota seed blower, USA) to remove dust and chaff from the seed-lot and graded with a laboratory sized clipper-cleaner (Blount Agri-Industrial, Indiana, USA). Seed for germination testing was counted using an electronic seed counter (Baumann Saatzuchtbedarf D-74638, Waldenburg, Germany). A standard germination test was developed based on International Seed Testing Association guidelines for *P. somniferum* (ISTA, 1999) and validated in a preliminary trial. Seeds were germinated either in a controlled temperature incubator or on a thermo-gradient table, depending on the experiment. All germination tests involved replicates of fifty seeds unless otherwise stated. Seeds were incubated in 9 cm Petri-dishes lined with two layers of Advantec No. 2 filter paper (Toyo Roshi Kaisher, Japan) for light and dark experiments. Seeds were assessed every 24 hours and the number of germinants recorded. Seeds were considered to be germinated when the radicle reached a length greater than 1mm. A „squash’ test was performed

at the end of the experiment to determine the condition of the un-germinated seeds (Yates *et al.*, 1996). Seeds with white or green embryos were considered as fresh non-germinated (Baskin and Baskin, 1998).

CONTROLLED TEMPERATURE INCUBATOR

Germination tests were conducted at 20°C ($\pm 1^\circ$) in a temperature controlled growth cabinet (Contherm, GPM). A replicate consisted of fifty seeds germinated in a single petri dish with two layers of Advantec No. 2 filter paper (Toyo Roshi Kaisher, Japan). The filter papers were initially moistened with 5 mL of distilled water. The petri dishes were checked every day, moisture levels observed and distilled water added as required.

THERMO-GRADIENT TABLE

Germination tests on a Terratec thermo-gradient table, 2 m long by 1 m wide, were performed within custom designed polycarbonate germination chambers (Plate 1) with a layer of Advantec No.1 filter paper saturated with distilled water. The germination chambers were constructed to permit solution in a reservoir to wick up onto the filter paper on which the seeds were placed. Each reservoir held up to 75 mL of the distilled water. A range of constant temperatures were obtained across the table. This was achieved by placing the germination chambers at regular intervals along the length of the table (Plate 2). Before commencing an experiment the temperature in each chamber was permitted to stabilise for a period of 24 hours. Temperature at the seed level was recorded over the duration of the study using thermocouples placed adjacent to seeds on the filter paper. Results confirmed that the temperatures remained within $\pm 1^\circ\text{C}$ of target temperatures throughout the experiments.



Plate 1: Custom designed cabinet made of Perspex. Each cabinet has 8 wells partitioned to hold water that can wick up onto the seeds. Each well can hold 75 mL of water.

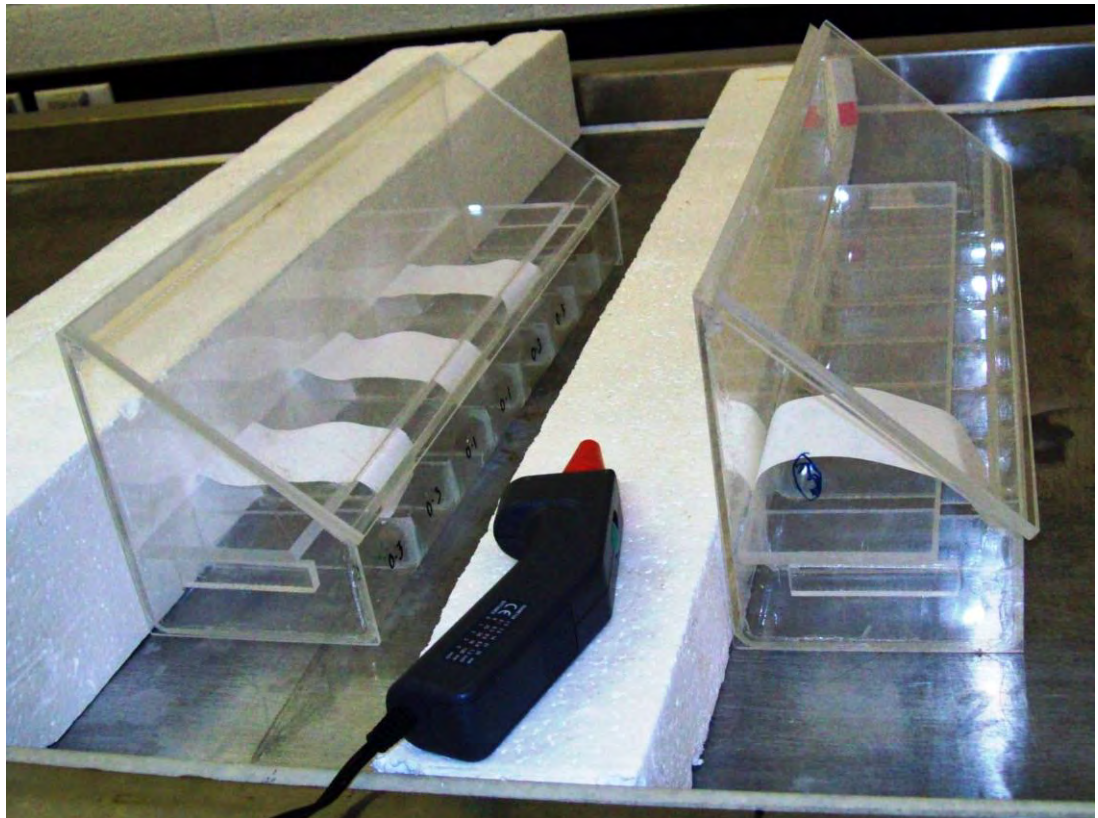


Plate 2: Perspex cabinets placed on the thermo-gradient table.

Plant Growth Facilities

Glasshouse and Shadehouse

Unless otherwise described, all plant growth experiments were conducted in the main glasshouse and in the shadehouse at the Horticultural Research Centre (HRC), University of Tasmania. Mean annual maximum/minimum temperatures were 25/15° C and 20/10° C. Daylength in Hobart varied from 16 monthly mean hours of daylight (sunrise to sunset) in December to 9 in June. Glasshouse daily maximum light levels ranged from 600 to 1500 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$. Shadehouse light levels ranged from 350 to 800 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$. Temperature was measured using a data logger (Tinytag Ultra) and light intensity was measured and logged using a line quantum sensor attached to a data logger (LI-191SA, LI-COR®, Biosciences, USA).

Plant growth and development experiments were conducted at the Horticultural Research Centre, University of Tasmania, Hobart (42° South, 147° East) using plants grown from the seed-lots described above. Plants were raised from graded seed germinated in trays filled with a moistened mix of 50% perlite, 25% coarse and 25% peat, a mix previously found to provide good plant establishment responses for small seeded species (Gracie *et al.*, 2003), and kept in controlled environmental conditions at a daily temperature of $20 \pm 3^\circ\text{C}$. Following plant emergence, seedlings were transplanted to individual pots. The potting mix used consisted of peat, sand and pine bark (1:2:7) and has a pH of 6.0. The fertilizer composition in the potting mixture was as follows: slow release (5-6 months) Osmocote® granules (330g/50L), dolomite lime (330g/50L), iron sulphate (25g/50L) and also trace elements (Micromax® 20g/50L). Nutrient solution (Hoagland's solution consisting of

Magnesium sulphate@200mL/litre; Calcium nitrate and Potassium nitrate @500mL/litre; Potassium di-hydrogen Phosphate @100mL/litre; and micro-nutrients Boric acid, Magnesium chloride, Zinc sulphate, Copper sulphate and Sodium Molybdate) was applied weekly after the „Osmocote’ capsules in the potting mix were exhausted. Over head irrigation three times a day was used for irrigation in experiments unless stated otherwise.

Plants were monitored regularly and for flowering experiments the date of anthesis recorded for each flowering plant. The plant height and number of fully mature leaves present on each plant was recorded on a weekly basis. Plant height was determined by measuring the distance from the base of the plant to the highest point of the rosette leaf arrangement. As stem elongation is negligible prior to flower stem development, plant height was essentially a measure of leaf length and the erectness of the leaves in the rosette structure. Leaf number included all fully expanded, mature leaves present on the plant but did not include leaves that senesced. Leaf number was therefore not a measure of the total leaf or node number on the plant.

Controlled Environment Chambers

Three controlled environment chambers or light tunnels at the HRC were used for vernalisation experiments. The chambers were constructed within the glasshouse structure and consisted of insulated, light proof rooms with a trolley system that could transfer plants between the glasshouse space and the controlled environment chamber. The light proof doors of each chamber were open when the trolley was in the glasshouse space and closed automatically when the trolley returned plants to the chamber. A computerised timing system controlled the movement of the trolley from

the glasshouse to the controlled environment chamber. Constant night temperature of 5° C was maintained using thermostatically controlled, refrigerated cooling elements. Artificial lighting within the tunnels was used where necessary to extend the natural daylength for long day treatments. Supplemental lighting was provided by combined mercury and fluorescent lights with a photon flux density of 30.2 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$. Light inside the controlled environment cabinets was provided by a mix of 400W GE Kolorarc MBID 400/T/H Tubular Metal Halide Lamps, (GE Hungary), 2 Osram Vialox Planta-T 400W sodium lamps, and 5 incandescent globes (100W Pearl; Osram, Germany). Due to space and facility constraints true replication was not achievable in transfer experiments, however continuous monitoring of the cabinets ensured that the temperature and photoperiod regimes remained constant.

PLANT CULTURAL PRACTICES

Following the first eighteen months of experimental work in the project, where several plant growth experiments were terminated due to the onset of severe leaf senescence and/or plant dormancy, a series of small trials were undertaken in an attempt to overcome the problem. Fungicide and miticide programs were developed to address pest and disease problems. One of the major problems encountered during the glasshouse experiments was white fly infestation. Hot temperatures prevailing inside the glasshouse coupled with hairy leaves of *P. bracteatum*, the white-fly infestation was most severe. The plants showed necrotic spots and other senescence symptoms. This problem was rectified by using a biological control agent *Encarsia formosa* to kill the larvae of white fly on the leaves. To control mite infestations, a predator (*Phytoseiulus persimilis*) was released to have a long term control than any chemical control. As the leaf chlorosis symptoms may have been caused by a

nutrient deficiency, an examination of effects of supplementary calcium nitrate nutrition was undertaken. Comparisons of plant growth under different irrigation systems were performed. Plants were placed on benches with either a capillary mat (Aquamat S10, Soleno Textiles, Quebec, Canada) or overhead sprinklers. Five plants were watered with overhead irrigation and a further five plants were placed on the capillary mats. Water was applied three times a day and sprinklers were set to turn on between 8 and 12 minutes while capillary mats were supplied with water between 5 and 8 minutes during each irrigation event. Observations from these trials were compiled (Table 1) and used to develop recommended plant cultural practices for glasshouse growth of *P. bracteatum*.

Table 1: List of agronomic practices followed during the whole length of the project in the glasshouse

Growing bags: Pot size	Observational results
6-8 inch pots	High proportion of plants become dormant in summer Stunted growth of plants except when grown under shading Plants prone to mite infestation Nutrient deficiency symptoms occasionally observed (chlorosis of older leaves)
16 litre pots	More vigorous growth of the plant Reduced frequency of dormancy and shorter period of dormancy Reduced nutrient deficiency symptoms
Irrigation system	
Over head sprinkler system	Necrotic lesions and wilting of flower stems and rotting of the capsules at maturity Severe mite infestations
Capillary mat system	Reduction in rotting of the capsules Reduction in the mite infestation
Fertilizer application	
Calcium Nitrate foliar spray	Reduction in the yellowing of leaves when grown in small pots Reduced leaf senescence when grown in small pots
Pesticide & Fungicide applications	
Fongafloor, Banrot, Sulphur	No reduction in leaf necrosis symptoms
Mancozeb, Kocide	No reduction in leaf necrosis symptoms
Pyranica, Procide, Eco-oil, Apollo, Omite, Clensel	Reduced infestation of thrips. Limited effectiveness against mite infestation unless combined with use of the predator <i>Phytoseiulus persimilis</i>
Biological control agents	
<i>Encarsia formosa</i>	Effective control of white fly
<i>Phytoseiulus persimilis</i>	Reduced incidence of mite infestations, but not effective control under all conditions

The best conditions for plant growth were found to be use of large 16L planter bags and capillary matting irrigation, combined with calcium nitrate foliar applications if leaf chlorosis symptoms were observed. Biological control agents are recommended for managing white fly and mites, with chemical treatments used under conditions favouring rapid development of mite populations.

STATISTICAL ANALYSIS

The designs of individual experiments are documented in the following chapters. For the majority of experiments, results were analysed with ANOVA using general linear model procedure of SPSS (V14.01) or the SAS (Statistical packages). For comparison of means, Fishers (Steel and Torrie, 1980) protected least significant difference (LSD) was calculated at 0.05 level of probability unless otherwise specified. Error bars shown in the graphs are standard errors of mean (SEM). Further details of experimental design, treatment of data and statistical analysis pertaining to individual experiments are given in the relevant experimental sections.

CHAPTER 3

SEED GERMINATION IN *PAPAVER BRACTEATUM*

INTRODUCTION

The potential of *Papaver bracteatum* as an economical source of thebaine, an alkaloid used for the production of opiates and other medicinally important compounds has been the catalyst for worldwide research on the species (WHO, 1980). Tasmania, Australia, is the only region in the southern hemisphere currently permitted to grow poppies for the licit medical opiates. Commercial interest from pharmaceutical companies on the medicinal value of *P. bracteatum* has led to its evaluation as a new crop in Tasmania.

The planting material used to establish preliminary field trials in Tasmania displayed characteristics typical of wild species, with uneven seedling emergence and low stand densities recorded (DPIWE, 2002). To overcome these challenges a greater understanding of seed dormancy and germination requirements is required. Ideally, crop establishment from seed will lead to even emergence of plants with uniform spacing between plants at the target density.

Seed germination is a process that commences with water uptake by the seeds and ends with the emergence of the radicle (Bewley, 1997; Bewley and Black, 1994). Temperature and water availability during imbibition have been widely reported to be the main factors that determine the level and rate of germination of non-dormant

seeds in laboratory tests and in field trials (Benech-Arnold and Sanchez, 1995). Under optimal moisture conditions, germination begins with rapid uptake of water driven primarily by seed matric potential. Small changes in Ψ_m (matric potential) have been shown to influence seed water uptake and germination rate to a much greater extent than changes in osmotic potential (Ψ_s) (Hadas and Russo, 1974). Water stress during germination may decrease or delay seedling emergence, reduce plant growth rate and over the growing season reduce crop biomass (Garwood, 1979; Huang, 1997). Sensitivity of small seeds to water stress during germination is particularly high because of limited availability of carbohydrate reserves (Billings, 1976). The range of temperatures over which a seed lot germinates is primarily dependant on the species, but is also influenced by seed lot quality and the degree of dormancy (Bewley, 1997).

In order to predict the performance of seed-lots in a cropping system, it is recommended that seed lots be assessed in terms of the degree of dormancy, level of viable seeds, uniformity of germination and capacity to germinate across a broad range of sub or supra-optimal conditions (Copeland and McDonald, 1995). In addition, the uniformity of germination and the time needed to reach maximum germination percentage also varies with temperature and water availability, and an understanding of these effects has led to the development of hydrothermal models to predict seed-lot performance (Gummerson, 1986). The capacity to predict germination at any temperature and water potential has the potential to direct management of establishment practices in order to optimise crop stands in the field.

To the knowledge of the author, few studies have been published on the seed germination of *P. bracteatum*. In their study, Bare *et al.* (1978) investigated the seed germination characteristics of three Papaver species; *P. bracteatum*, *P. orientale* and *P. somniferum* under different light and temperature regimes. The maximum germination level of *P. bracteatum* seed was achieved at temperatures ranging from 18° to 26°C and germination rate occurred at temperatures ranging from 21° to 26°C. This was a narrower optimal temperature range than that recorded for *P. orientale* and *P. somniferum*, with the maximum germination rate recorded for the two species ranging from 18° to 30°C and 13° to 33°C respectively. In the same study Bare *et al.* (1978) reported no germination above 30°C and when dormancy was induced in *P. bracteatum* seeds germinated when exposed to 35°C pre-treatment far red light and red light promoted germination to the same level. The same response was not recorded at lower temperatures with seed able to germinate under both light and dark conditions. In *Papaver rhoeas* the degree of dormancy decreases during warm periods and increases during cool periods (Baskin *et al.*, 2002; Cirujeda *et al.*, 2006; Karlsson and Milberg, 2007; Milberg and Andersson, 1997). In field conditions, *Papaver argemone*, *Papaver rhoeas*, and *Papaver dubium* were reported to germinate over several seasons irrespective of prevailing climate and weather conditions (Karlsson and Milberg, 2007), suggesting a morpho-physiological dormancy mechanism. The nature and extent of dormancy in *P. bracteatum* needs to be confirmed as it has the potential to impact significantly on crop establishment from seed. This study addresses the paucity of understanding of the germination requirements of *P. bracteatum* and investigated the cardinal temperatures for germination, light and water requirements, and the loss in viability during storage.

MATERIALS AND METHODS

SEED SOURCE

Seed lots were obtained from *P. bracteatum* plants grown under glasshouse or shade house conditions at the Horticultural Research Centre located at the Sandy Bay campus of the University of Tasmania. Capsules were harvested, dried cleaned as outlined in chapter 2. A series of experiments were undertaken to determine the light, temperature, water potential requirements for germination, and effects of short-term storage on germination.

LIGHT REQUIREMENT

Light requirement for the germination of *P. bracteatum* seed was assessed by comparing three different seed sources ranging from freshly harvested to long-term stored. Seed-lots were denoted A (stored for 26 weeks), B (stored for 52 weeks) and C (freshly harvested). Each seed-lot was imbibed under either continuous light or continuous dark. Four replicates of 50 seed of each treatment (light and dark) were used and were randomly arranged within a controlled temperature cabinet set to 20°C. Seeds were germinated in petri-dishes sealed with Parafilm (Parafilm® Model 60631, CHICAGO, IL, (101.6 mm) to limit moisture loss. The quality and quantity of light used in the light and dark experiments was by using fluorescent lamps (GE Tri-Tech F36T8/840) which produced 1200-1500 k and 330 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ respectively. Dark treatment was achieved by individually wrapping petri-dishes with two layers of aluminium foil. The number of germinated seeds was recorded on a daily basis for a period of fourteen days. Seeds germinated in dark conditions were checked under

green safe light. When necessary, distilled water was added to petri-dishes during germination assessments to maintain adequate moisture levels.

EFFECT OF STORAGE

The effect of storage time on germination percentage was assessed using a graded and an ungraded seed-lot, and the rate of loss of viability during storage was calculated from the germination data. The first seed lot, harvested in 2006, was cleaned but not size or density graded and the second seed lot, collected in 2007, was density graded by air-screening (South Dakota seed blower, USA) and size graded with a laboratory sized clipper-cleaner (Blount Agri-Industrial, Indiana, USA). Seed in the median density, median size class was used in the study. Seed lots were stored in double plastic bags at 20°C for 36 months. Every month, commencing shortly after seed was harvested from the mother plants, a sub-sample of seeds was taken from the bulk seed lot and germinated at 20°C at 12:12 light in a controlled temperature cabinets. Four replicates of fifty seeds were used in each assessment.

EFFECT OF TEMPERATURE

The experiment was conducted to investigate the germination characteristics of *P. bracteatum* seed lots under a range of constant temperatures. Eight constant temperatures ranging from 5° to 35°C were obtained on the thermo-gradient table (Refer Chapter 2). Two separate seed lots from the plants grown under glasshouse conditions from two different seasons (2006, stored for 2 years, and 2007, stored for 1 year) were used. Four replicates of 50 seeds from each of the two seed lots were

germinated at each temperature. Radicle emergence was assessed every 24 h over a period of 28 days.

TEMPERATURE AND WATER AVAILABILITY

The interaction between temperature and water potential on germination response of *P. bracteatum* seed was investigated. The experiment consisted of four constant temperatures (9°, 15°, 20° and 30°C) and three water potentials (0, -0.1, -0.3 MPa). -0.1 and -0.3 MPa water potential solutions were prepared using aqueous solutions of polyethylene glycol (PEG 8000) according to Michel (1983). The ψ - values of the solutions were checked using a vapour pressure osmometer (Model 5100 c; Wescor Inc., Logan, UT, USA), which was calibrated using NaCl standards, and corrected for each constant temperature (Michel and Kaufmann, 1973). Custom built germination cabinets kept on a thermo-gradient table were used. Four replicates of each water potential treatments were randomly allocated to chambers at each temperature. The seeds were placed on the filter paper inside the cabinets and the PEG solution was permitted to wick on to the filter paper, maintaining the seeds at constant water potential. Seeds were transferred every twenty four hours across to fresh chambers to ensure they were exposed to constant water potential in the cabinets over the duration of the experiment. Regular assessment of solution ψ - on filter paper sections using a vapour pressure osmometer confirmed that water potential remained relatively constant for the duration of the experiment. The number of seeds that germinated was recorded daily until 21 days after imbibition.

DATA ANALYSIS

All statistical analyses were performed using SAS version 9.1 (SAS, Institute, Cary North Carolina, USA) statistical package. SAS procedure NLIN was used to describe the time course of germination of individual replicates of fifty seeds using the logistic growth curve equation:

$$Y_t = M [1 + \exp (-K*(t - L))]^{-1}$$

Where; Y_t is the cumulative percentage germination at time t , M is the asymptote (theoretical maximum for Y_t), K is the proportional to the rate of germination, and L is the time to 50% maximum germination, M . The logistic function described above has been widely used in seed germination studies to describe the time course of germination (Dumur *et al.*, 1990; Shafii *et al.*, 1991; Thompson *et al.*, 1994). Data recorded in the light and dark, effect of temperature and effect of water potential experiments were analysed using the GLM procedure to test treatment effects on the maximum germination and time to 50% germination (T-50) consistent with the experimental design. Proc REG was used to determine the relationship between seed viability and duration of storage. Percentage germination data were arcsine square-root transformed to meet the assumptions of normality and homoscedasticity prior to analysis.

RESULTS

LIGHT AND DARK:

No significant differences were found in the maximum percentage germination of *P. bracteatum* seeds imbibed under continuous light or dark at 20°C. Differences in germination percentage were noted between seed-lots, with germination percent nearly 5% higher both in light and dark for the older seed lot (52 weeks storage).

Table 2: Germination percentage of *P. bracteatum* seeds 7 and 14 days after imbibition (DAI) at 20°C in light or dark. Each value is a mean of four replicates (\pm SEM).

Seed-lot	DAI	Light (%)	Dark (%)
A (26 weeks)	7	63.3 \pm 1.77	62.6 \pm 1.34
	14	76.6 \pm 0.67	71.3 \pm 1.77
B (52 Weeks)	7	71.3 \pm 2.91	67.3 \pm 1.77
	14	84.0 \pm 1.16	80.0 \pm 1.16
C (Fresh seed)	7	63.3 \pm 1.77	58.6 \pm 1.78
	14	80.0 \pm 3.53	82.0 \pm 1.77

STORAGE DURATION:

The proportion of viable seeds in both the graded and ungraded seed lots decreased at relatively constant rates when stored at 20°C (Fig 1). The graded seed-lot had a significantly higher initial germination percentage ($89 \pm 2.9\%$) than the ungraded seed-lot ($70 \pm 1.9\%$). The rate of decrease in proportion of viable seeds per week was calculated using the slope of the germination percentage versus time plot, with variability determined at the 95% confidence interval. The rate of decrease in proportion of viable seeds per week was significantly higher for the ungraded seed-lot ($0.22\% \pm 0.023$), than the graded seed-lot ($0.13\% \pm 0.020$).

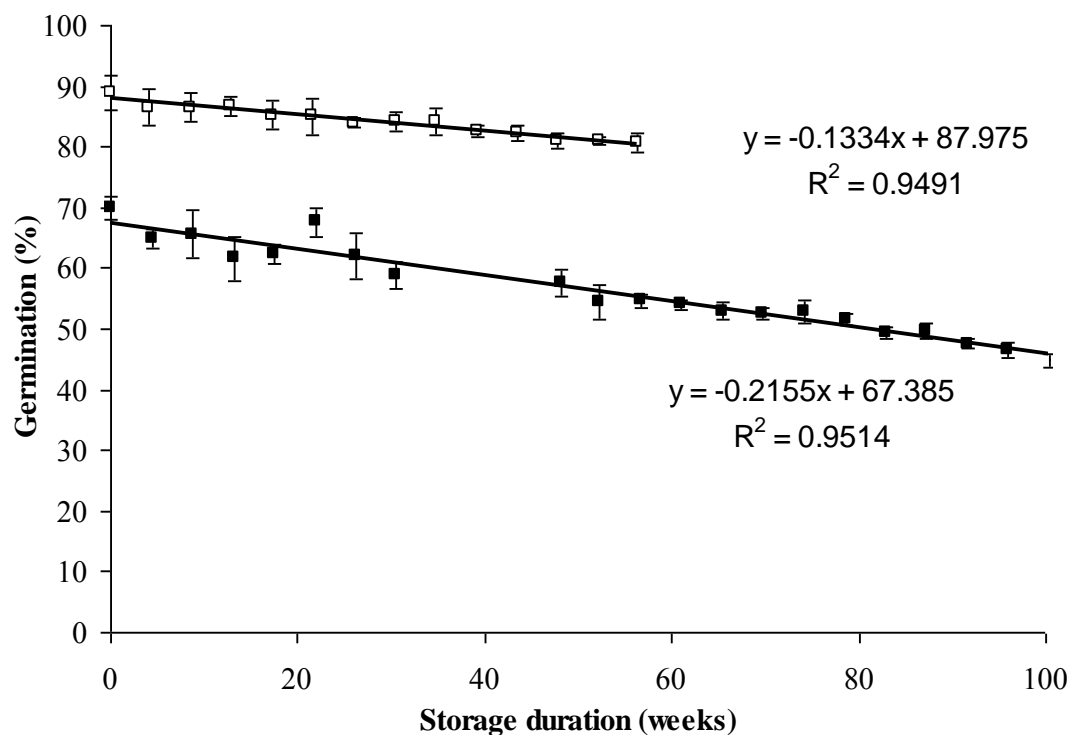
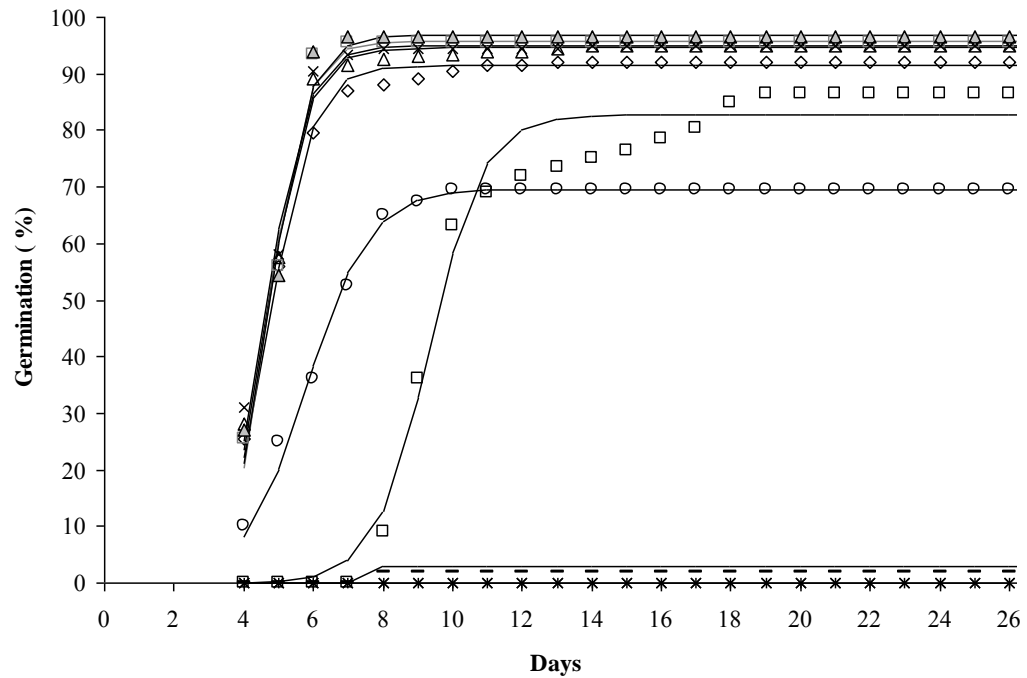


Figure 1: The effect of storage duration (weeks) on the germination of ungraded (■) and graded (□) *P. bracteatum* seed. Seeds were stored at 20°C and each point represents the mean of four replicates of fifty seeds \pm SEM. The slope and intercept of the regression lines for the two seed lots are significantly different ($p < 0.001$).

EFFECT OF TEMPERATURE

Logistic regressions fitted to the data provided a good fit for both seed-lots across a wide range of temperatures assessed for one year and two year old seed lots (Figure 2). The maximum germination percentage and time to 50% germination (t-50) were derived from the fitted model. No significant difference was recorded in the maximum germination and rate of germination for temperatures ranging from 15° to 25°C for both seed-lots (Figure 2). However, there was a significant difference in germination rate and level between this optima range (15-25°C) and temperatures higher and lower than this for both the one-year ($F_{7, 24} = 248.16$, $P < 0.0001$) and two-year old seed-lots ($F_{7, 24} = 203.511$, $P < 0.0001$). While both seed lots responded similarly across the optima temperature range, a difference in response was recorded at 30°C; germination percentage of approximately 85% was recorded in the two year old seed-lot, while it was only 70% in the one year old seed-lot (Figure 2). The one-year seed-lot also exhibited a slightly higher germination level and lower t-50 at 9°C compared with the two-year old seed-lot suggesting that it was slightly more sensitive to supra-optimal temperature and less sensitive to sub-optimal temperature stress.

(a)



(b)

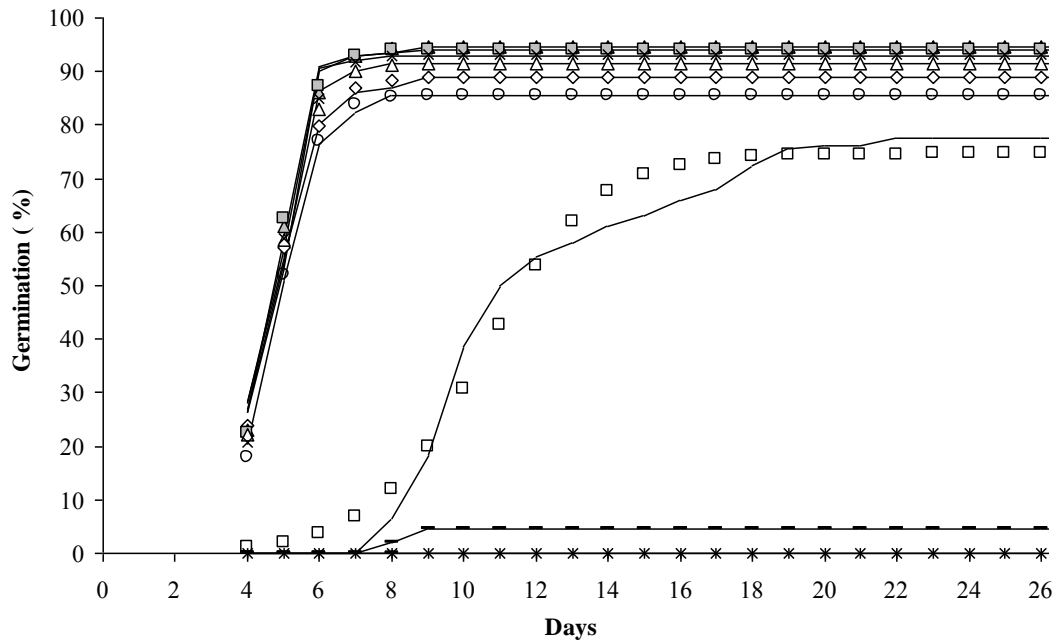
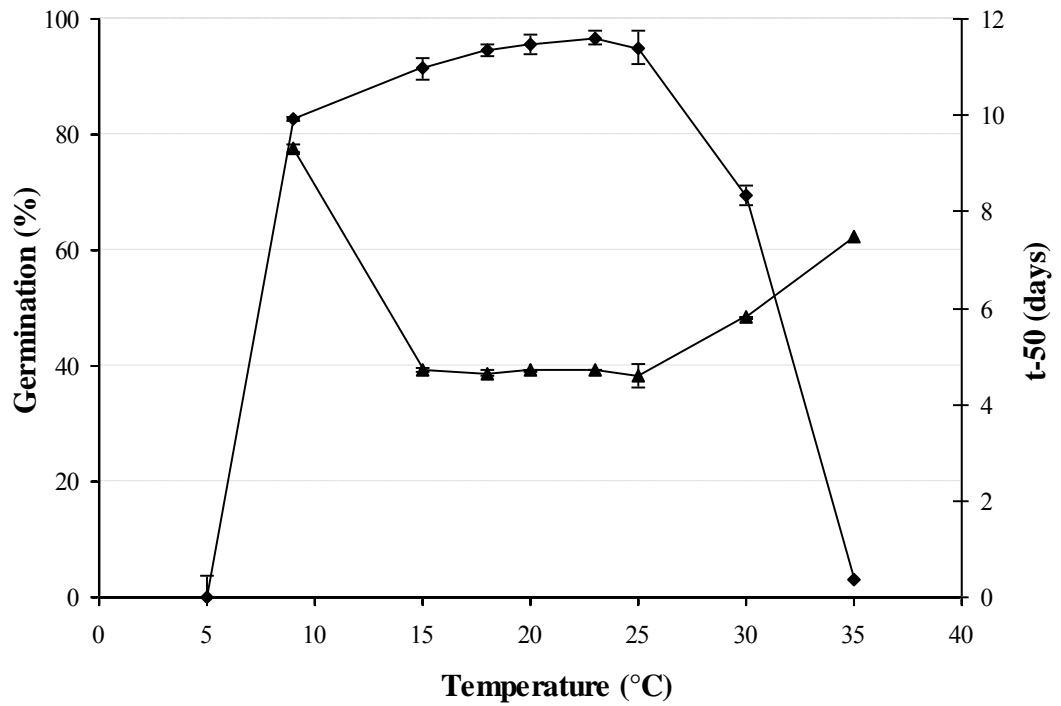


Figure 2: Cumulative germination of (a) one and (b) two year old seed lots of *P. bracteatum* over a period of 28 days at different temperature regimes. Data points are means of 4 replicates of 50 seeds. Symbols represent (*) = 5°C; (□) = 9°C; (◇) = 15°C; (▲) = 18°C; (■) = 20°C; (▼) = 23°C; (×) = 25°C; (○) = 30°C; (—) = 35°C. Points are the observed data and solid lines indicate the fitted curves.

(a)



(b)

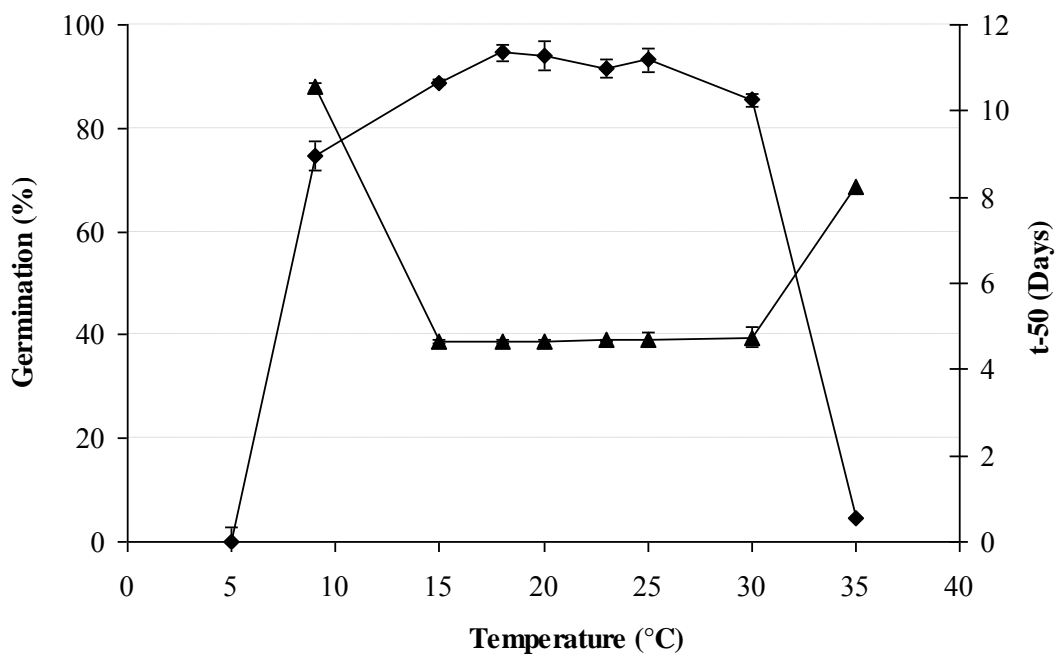


Figure 3: Maximum of germination (%) (♦) and time taken (days) to fifty percent germination (t-50) (▲) of one (a) and two (b) year old seed lots of *Papaver bracteatum* germinated at a range of constant temperatures. Bars represent SEM (n=4).

WATER POTENTIAL:

Germination was completely inhibited at water potentials of -0.1 and -0.3 MPa at temperatures of 20°C or higher. Only approximately 20% of the seeds were able to germinate at -0.1 and -0.3 at 9°C and 15% at 15°C.

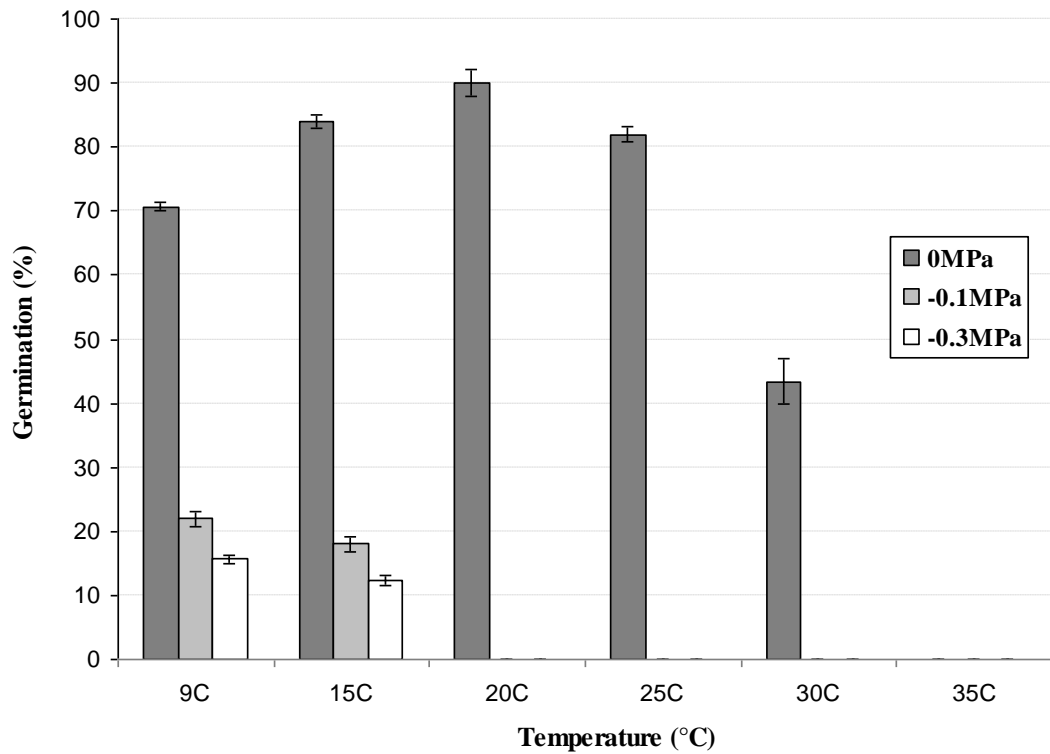


Figure 4: Percentage germination of *P. bracteatum* seeds at different water potentials (0; -0.1; -0.3 MPa). Each value is a mean of three replicates of fifty seeds. Bars represent SEM (n=4).

DISCUSSION

No physiological dormancy was detected in freshly harvested *P. bracteatum* seeds, regardless of the growing conditions of the mother plant; similarly, no physiological dormancy was detected in seeds stored for up to 3 years. Although thermal induced secondary dormancy has been reported in *P. bracteatum* at high temperatures (Bare *et al.*, 1978), this response was not confirmed in the present study. However, the germination response of seeds at supra-optimal temperatures was in general agreement with Bare *et al.* (1978) who reported a decrease in germination at 27°C and no germination at 32°C. In the present study, a small proportion (<5%) of seeds germinated at 35°C.

No previous studies have been conducted on the effect of water potential and the results of this present study showed that *P. bracteatum* seeds were sensitive to water deficits. This sensitivity was exacerbated at high temperature. *P. bracteatum* originates from temperate, mountainous regions of Iran (Sharghi and Lalezari, 1967) where high temperatures and low rainfall are common in summer and was previously reported to be drought resistant (Neild, 1987). The inability of *P. bracteatum* seeds to germinate at low water potentials and high temperatures suggests that, while established plants may be able to survive under drought conditions, the capacity to establish crops from seed in areas with insufficient soil moisture will be limited. The slow rate of germination, sensitivity to low water potential and the low proportion of seeds that germinate at low temperatures are considered constraints to industry flexibility in sowing time and site selection in cooler-temperate regions, such as Tasmania. As capacity to germinate at low temperatures is an important attribute in

cool temperate regions, seed sowing during warmer months or seed priming should be considered for future seed evaluations.

A loss of seed viability with increasing duration of storage of between 0.1 and 0.3% per week over a 3 year period was recorded in *P. bracteatum* with a poor quality, ungraded seed lot losing viability at a higher rate than the high quality, graded seed lot. It was concluded that seeds of *P. bracteatum* could be stored for a period of nine months at 20°C with a slight decrease in viability, and for up to two years with a reduction in germination percentage of around 20%. These results demonstrate that adequate maintenance of seed quality for commercial plantings may be obtained without specialised storage treatments, with scope for longer term storage but further research would be needed to identify optimum storage conditions. Low temperature and low seed moisture are the two effective means of maintaining seed quality in storage (Bonner, 2003).

Bare *et al.* (1978) concluded that the optimal temperature to achieve maximum germination percent was between 18° and 26°C. The optimal germination temperature range of 15°C to 25°C recorded in the present study is therefore consistent with the previous study. The highest germination percentage and rate of germination, recorded as time taken to reach 50% germination (T50), was 23°C and germination was inhibited at temperatures of 5°C and 35°C. Previously no germination was reported below 10°C by Bare *et al.* (1978). The knowledge of the germination characteristics of *P. bracteatum* seeds will assist the Tasmanian industry to select and manage establishment conditions for the crop.

CHAPTER 4

SEED QUALITY IN *PAPAVER BRACTEATUM*

INTRODUCTION

Sustainable commercial production of *Papaver bracteatum* in Tasmania will benefit from the development of guidelines for successful cultivation of the crop. Production guidelines should include recommendations for achieving uniform stands of seedlings at the target density given that, for a range of crops, yield has been shown to be strongly influenced by the success of the establishment phase (Clark, 2002). The establishment phase is largely influenced by two main factors; the planting environment and the quality of the seed used (Gray *et al.*, 1991; Hegarty, 1978; Tamet *et al.*, 1996).

Seed-lot quality can be defined as the ability of a seed-lot to produce rapid and uniform field emergence of healthy seedlings, and store for long periods without losing viability (Hampton and TeKrony, 1995; Valdes and Gray, 1998). Seed quality is therefore a broad term that includes seed viability, vigour, genetic purity and seedling development (Cardwell, 1984; Ellis, 1992; Ellis and Roberts, 1980; Esbo, 1980). The need for high quality seeds has been recognised useful for production systems that rely on transplants and also for those using direct seeding (Jett *et al.*, 1996; McCormac and Keefe, 1990).

For trade purposes, seed-lots are characterised by the proportion of seeds that germinate to produce seedlings within a given period under ideal, species specific, conditions (ISTA, 1999). However, a knowledge of the proportion of viable seeds within a seed-lot alone is regarded by seed users as being limited (Harrington, 1972) since it does not provide a full understanding of how seed-lots will perform under sub-optimal conditions nor of rate and uniformity of germination. Hence, a single measure of germination capacity does not necessarily provide reliable information on seed quality and seed-lots displaying equal germination capacity in standard tests may perform quite differently in the field (Perry *et al.*, 1990; TeKrony and Egli, 1991).

In addition to the standard germination tests (ISTA, 1999) a number of seed-lot quality tests have been devised as an assessment of seed-lot quality. These tests generally assess seed vigour and help in identifying poor and good seed lots in a short period of time and correlate stress conditions under which plants are grown in the field (Trawatha *et al.*, 1990). Seed quality tests include amongst others laboratory tests at sub-optimal temperatures, electrical conductivity and tetrazolium viability tests (Kolasinska *et al.*, 2000).

The production of high quality seed can be challenging. Studies have demonstrated that the quality of a seed-lot can be affected by environmental and agronomic factors during seed development on the maternal parent (Ghassemi-Golezani, 1992), harvesting time and techniques (Oplinger *et al.*, 1989), post-harvest handling (Elias and Copeland, 2001), and storage conditions and duration (Adebisi and Ajala, 2007; Adebisi and Ojo, 2001; McDonald, 2000; TeKrony *et al.*, 1980; Tesnier *et al.*, 2002).

However, obtaining high quality seed at the point of harvest from the maternal parent is regarded as the most vital stage (Wellington, 1969). A study by Oplinger *et al.* (1989) demonstrated that harvesting *Brassica napus* (canola) seed too early, when the seed is still immature, resulted in seed-lots with low levels of viability or poor vigour, whereas harvesting too late resulted in seed shattering and reduced yield. Therefore, the optimum time to harvest seed requires a careful balance between maximising seed quality parameters while minimising potential seed loss. Confounding this is that, under commercial conditions, when all the plants in the field are harvested at the same time, not all of the harvested seeds may be of the same physiological maturity, as reported in carrot by Hawthorne *et al.* (1962).

It has been recommended that harvesting of a seed crop should commence soon after achieving the maximum seed quality (Siddique and Wright, 2003), even though most seeds are capable of germinating long before physiological maturity (Galau *et al.*, 1991; Harrington, 1959; Hill and Watkin, 1975; Pegler, 1976; Rasyad *et al.*, 1990). Harrington (1972) proposed a hypothesis that seeds attain maximum seed quality at the end of the seed-filling phase and eventually lose viability and vigour as they age. Findings by Sanhewe and Ellis (1996) contradicted the previous hypothesis and demonstrated that maximum seed quality was not attained until some time after the end of the seed-filling phase in *Phaseolus vulgaris* (Bean).

In many studies, indicators of seed maturity, such as seed moisture content (Hill and Watkin, 1975; Steiner and Akintobi, 1986), and seed quality, such as size of the seed (Ellis *et al.*, 1993), have been proposed for determining the time of harvest seed. For example, Gray and Steckel (1982) were able to demonstrate, within genotype, a link

between the optimum time to harvest seed in *Daucus carota* L. (carrot) and seed size and seed moisture. Similar links between seed size and harvest time have been recorded in *Lycopersicon esculentum* L. (Tomato) (Demir *et al.*, 2008), members of the *Brassica oleracea* family (Borthwick, 1931) and in *Allium cepa* L. (onion) (Spurr, 2003). However, the use of harvest indicators is often species specific and has required an in-depth understanding of the physiological development of the seed on the mother plant.

In addition to optimising time of harvest there are a range of post-harvest techniques used to improve the quality of a seed lot. These techniques include seed grading, priming, pre-soaking, growth regulator application, stratification, scarification, aeration, hardening, and coating with pesticides, nutrients or microbes (Khan, 1992). Seed grading is one of the easiest and quickest approaches to improve the quality of a seed-lot and generally involves separating the seeds based on size or density. The larger and denser seed within a seed lot often have better seed germination characteristics than the small, lighter seed (Davidson *et al.*, 1996; Toon *et al.*, 1990). Studies on forest tree species (Ke and Werger, 1999; Navarro *et al.*, 2006; Singh *et al.*, 1993), and vegetable crops; lettuce, *Lactuca sativa*, (Wurr and Fellows, 1983), carrot, *Daucus carota* (Gray and Steckel, 1983), and onions (Milberg *et al.*, 1996; Weis, 1982; Wulff, 1986), have all reported a positive correlation between size of the seed and germination percentage. Although a negative correlation between these parameters has previously been reported for some of the wild species such as *Quercus libani*, *Tectona grandis* (Alptekin and Tilki, 2002; Indira *et al.*, 2000; Shepard *et al.*, 1989) these negative responses are very rare and in most cropped

species seedling establishment, growth and yield are effected by seed size, leading to grading of seed for commercial use.

The effect of time of harvest and seed grading on seed quality in *P. bractetatum* have not been studied previously. Knowledge on of this valuable information has the potential to contribute significantly in the development of production guidelines for establishment of commercial crops of *P. bractetatum* in Tasmania, Australia.

MATERIALS AND METHODS

Seeds used in this work were harvested from mature plants grown in shadehouse conditions at Horticulture Research Centre (HRC), University of Tasmania (S 42° 54.306', E 147° 19.467' at an elevation of 55m). Plants were grown in 16 litre pots. The fertilizer composition in the potting mixture as follows: slow release (5-6 months) Osmocote® granules (330g/50L), dolomite lime (330g/50L), iron sulphate (25g/50L) and trace elements (Micromax® 20g/50L). Irrigation was provided three times a day using drippers on to each individual pot to prevent disease infections caused by overhead irrigation system. Nutrient solution (Hoagland's) was applied weekly after the Osmocote® capsules in the potting mix were exhausted. Two experiments were conducted in this study; the optimal time to harvest capsules, and the use of seed grading to improve seed-lot quality.

TIME OF HARVEST

Twenty plants of relatively uniform size were selected from a larger population and used in this trial. Prior to the commencement of flowering, each plant was randomly assigned to one of five harvest times; 14, 28, 42, 56 and 70 days after full bloom (DAFB). Flowering commenced in November, 2008 and ceased in January, 2009. Flowers on the primary and secondary stems were tagged when they reached full bloom. When the allocated period from full bloom of the primary stem for individual plants had elapsed the capsules were cut just below the thalamus, weighed and placed into brown paper bags for drying. Drying procedures were outlined in chapter 2 and dry weights of the capsules were recorded, hand threshed and seeds were partially cleaned using hand sieves to remove coarse trash and fine dust, and later

manually separated. The number and weight of seeds were recorded for each capsule. A sub-sample of 100 seeds from each seed lot was used to determine seed moisture content by oven drying at 110°C for a period of two days. A second sub-lot was used to determine the germination characteristics.

Seeds were germinated in accordance with ISTA guidelines (ISTA, 1999). Four lots of 50 seeds from each capsule were germinated at 20°C and at 10°C. Seeds were germinated in a custom designed germination cabinet (Refer chapter 3) with a layer of Advantec No.2 filter paper saturated with distilled water. The number of seeds that germinated each day was recorded. Seeds were considered to be germinated when the radicle reached a length of at least 1mm. The germination tests were completed in three weeks. A „squash’ test was performed at the end of the experiment to determine the viability of the un-germinated seeds (Yates *et al.*, 1996). Seeds with white or green embryos were considered as viable (Baskin and Baskin, 1998).

SEED GRADING

Capsules harvested from a population of plants, grown under shadehouse conditions, were sun dried for a period of two weeks and cleaned to remove non-seed material using a laboratory sized clipper cleaner (Blount Agri-Industrial, Indiana, USA). The cleaned seed-lot was then graded by density into three density classes (light, medium and heavy) using a perforated air column (South Dakota Seed blower, Seedburo, USA), to produce three sub-lots containing approximately the same number of seeds. Each density class was graded by size into three size classes (small, medium and large) to produce a total of 9 sub-lots containing an approximately even number of seeds in each. Seeds were graded by size using a set of stacked square mesh screens

(Swiss screens, Switzerland) declining sequentially in aperture size (Figure 5). Seeds less than 630 μm were classified as 'small', between 630 and 670 μm as „medium' and more than 670 μm as „large'. Cleaned dry seed lots were stored in sealed bags in the dark at 20°C. Three replicates of 40 seeds from each size by density grouping were imbibed at 20°C and 10°C and germination recorded as described for the time of harvest trial (above).

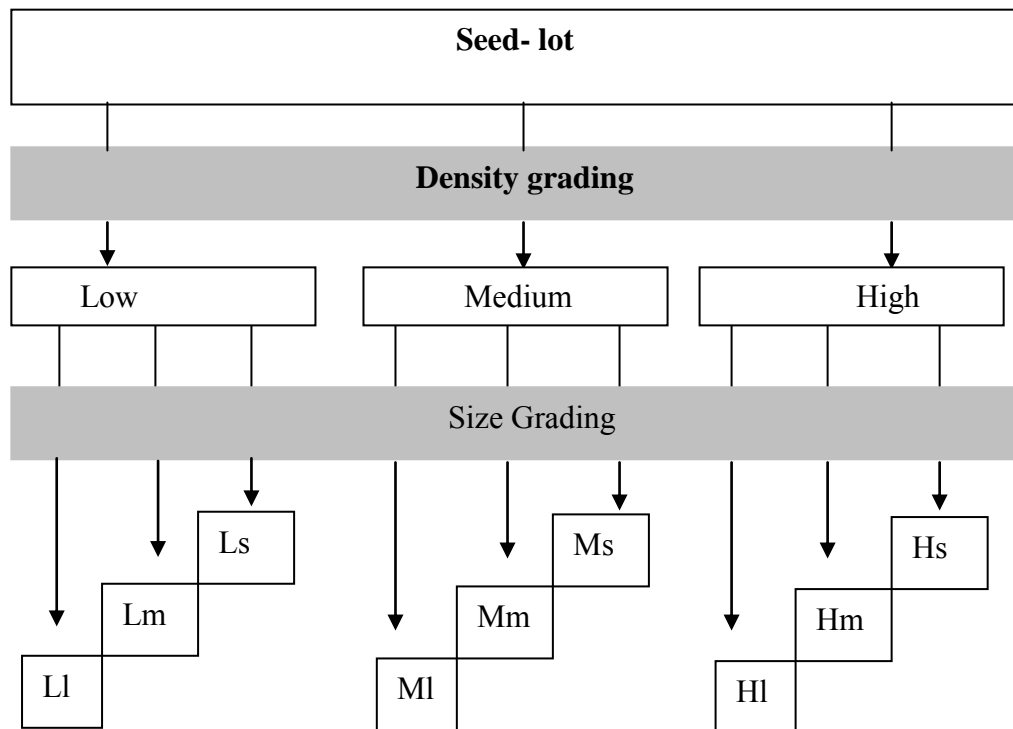


Figure 5: *Papaver bracteatum* seed-lots were graded by density into Low (L), Medium (M) & High (H) density groups and then by 3 sizes; Small (s), Medium (m) and Large (l).

STATISTICAL ANALYSIS

All statistical analyses were performed using SAS version 9.1 (SAS, Institute, Cary North Carolina, USA) statistical package. SAS procedure NLIN was used to describe the time course of germination of individual replicates of fifty seeds using the logistic growth curve equation:

$$Y_t = M [1 + \exp (-K*(t - L))]^{-1}$$

Where; Y_t is the cumulative percentage germination at time t , M is the asymptote (theoretical maximum for Y_t), K is the proportional to the rate of germination, and L is the time to 50% maximum germination. The logistic function described above has been widely used in seed germination studies to describe the time course of germination (Dumur *et al.*, 1990; Shafii *et al.*, 1991; Thompson *et al.*, 1994). Co-efficient of uniformity of germination (CUG) was calculated to provide a measure of spread in germination and is expressed as a variance of individual times around the mean time to complete germination. It is calculated by the formula:

$$CUG = \Sigma n / \Sigma [(MGT - t_x)^2 . n]$$

where; t_x is the time in days starting from day zero as day of wetting; n is the number of seeds germinating on day x ; and MTG is the mean time to complete germination, which is an average measure of the time taken by individual seeds to germinate.

Data recorded from both the seed grading and time of harvest experiment were analysed using the GLM procedure to test treatment effects on the maximum germination, time to 50% germination (T50) and CUG. In the time of harvest experiment each plant was considered a replicate, whereas each sub-lot of 40 seeds in the seed grading trial was regarded as a replicate. Percentage germination data were arcsin square-root transformed and T50 data were log transformed to meet the assumptions of normality and homoscedasticity prior to analysis.

RESULTS

TIME OF HARVEST

The mean dry weight of 100 seeds increased from 10.8 mg at 14 DAFB to 30.5 mg at 70 DAFB (Figure 6). The rate of increase in weight from 14 DAFB to 42 DAFB was approximately 1.78 mg/week, with only a small increase from 42 to 56 DAFB before increasing by 12.5 mg /week from 56 to 70 DAFB (Figure 6).

The time of harvest had a significant effect on the percentage of seeds that germinated ($F_{4,15} = 72.15$; $P < 0.001$) but not the rate of germination ($F_{4,15} = 1.34$; $P = 0.301$), expressed as the time to 50% germination, when imbibed at 20°C in accordance with ISTA guidelines (Figure 7). The percentage of seeds that germinated increased from 42% when harvested at 14 DAFB to 88% at 56 DAFB (Figure 7). No significant difference was detected between seeds harvested 56 (88%) and 70 (90%) DAFB. The mean time to 50% germination across harvested dates was approximately 7 days.

When the seeds were imbibed at 10°C a significant difference in maximum percent germination ($F_{4,15} = 232.44$; $P < 0.001$) and the time to 50% germination ($F_{4,15} = 4.39$; $P = 0.015$) was recorded. The percentage of seeds that germinated increased from 18% in seed-lots harvested at 14 DAFB to 90% at 70 DAFB (Figure 7). In contrast to imbibition at 20°C, a significant difference was detected between seeds harvested 56 (80%) and 70 (90%) DAFB due to a lower proportion of seeds germinating in the 56 DAFB treatment. A significant difference was observed between harvest dates on the time to 50% germination at 10°C. There was a general decrease in T50 with later harvests with the first harvest date (14 DAFB) having the highest T50 and the last

harvest (70 DAFB) having the lowest t50 (Figure 7). At 10°C the time taken to T50 germination was 11 days and at 20°C it was only 7 days. The Coefficient of uniformity of germination, a measure of spread in germination around the mean time to complete germination, was higher at 10°C than 20°C (Figure 8).

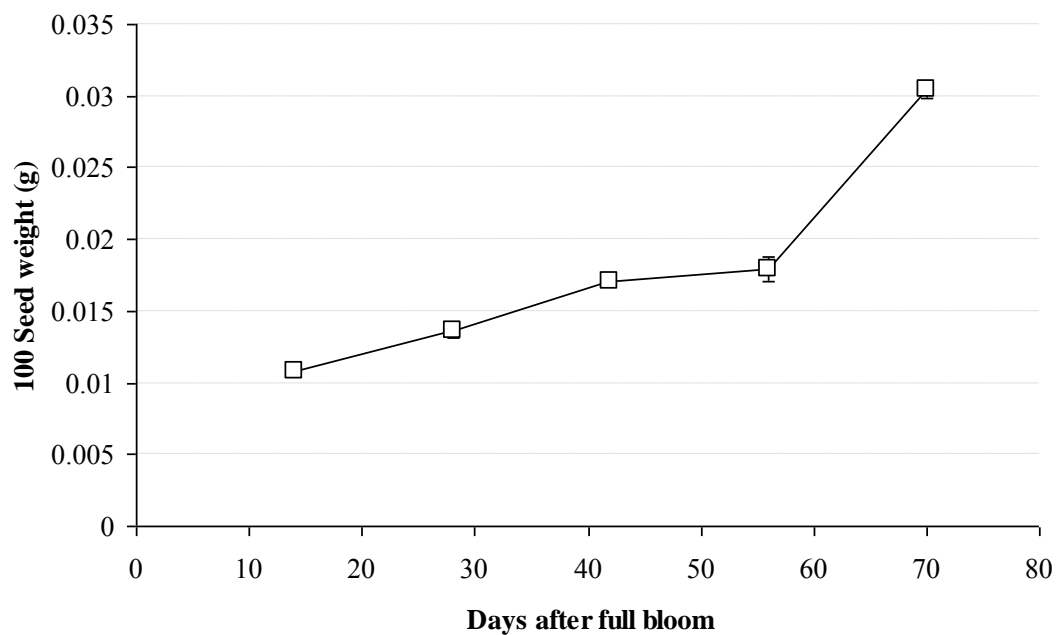
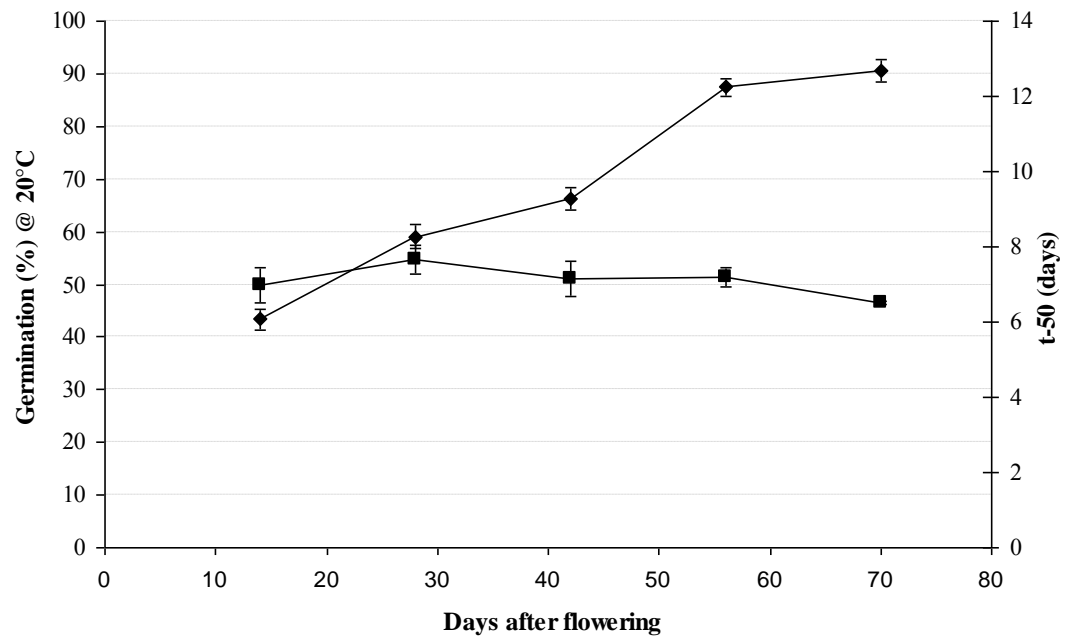


Figure 6: Hundred seed dry weight (g) of *P. bracteatum* seeds. Data points are means of 4 replicates of 100 seeds \pm SEM.

(a)



(b)

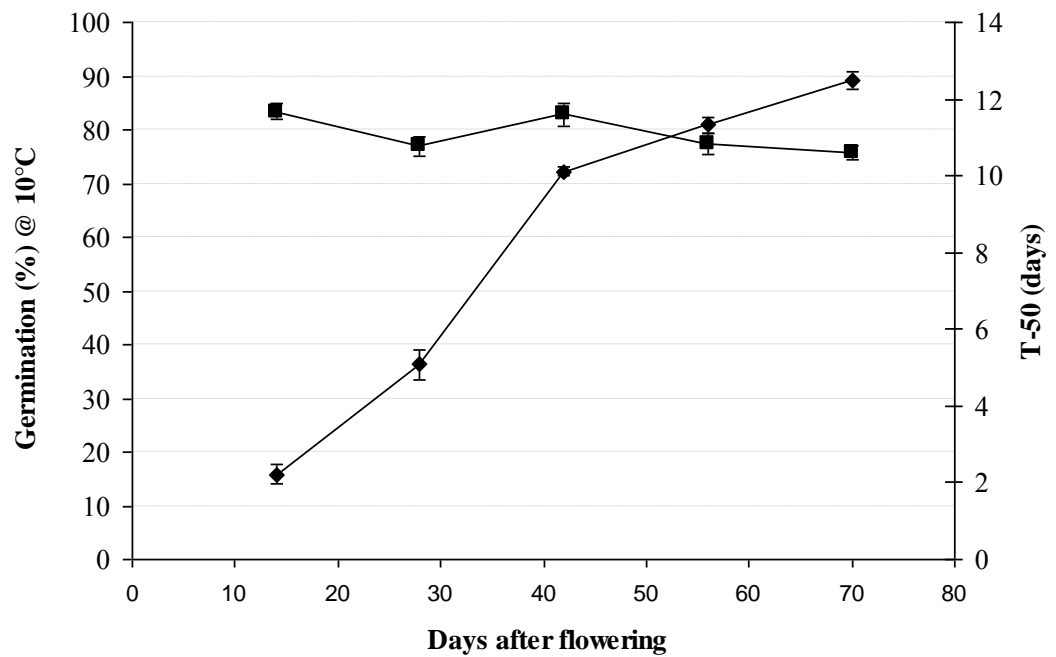


Figure 7: Maximum germination percentage (♦) of *Papaver bracteatum* seed 21 days after imbibition at (a) 20° and (b) 10°C. Seeds were harvested 14, 28, 42, 56 and 70 days after flowering. Data points are means of 4 replicates of 50 seeds \pm SEM. Secondary axis represents the time taken to reach t-50 (■) (days).

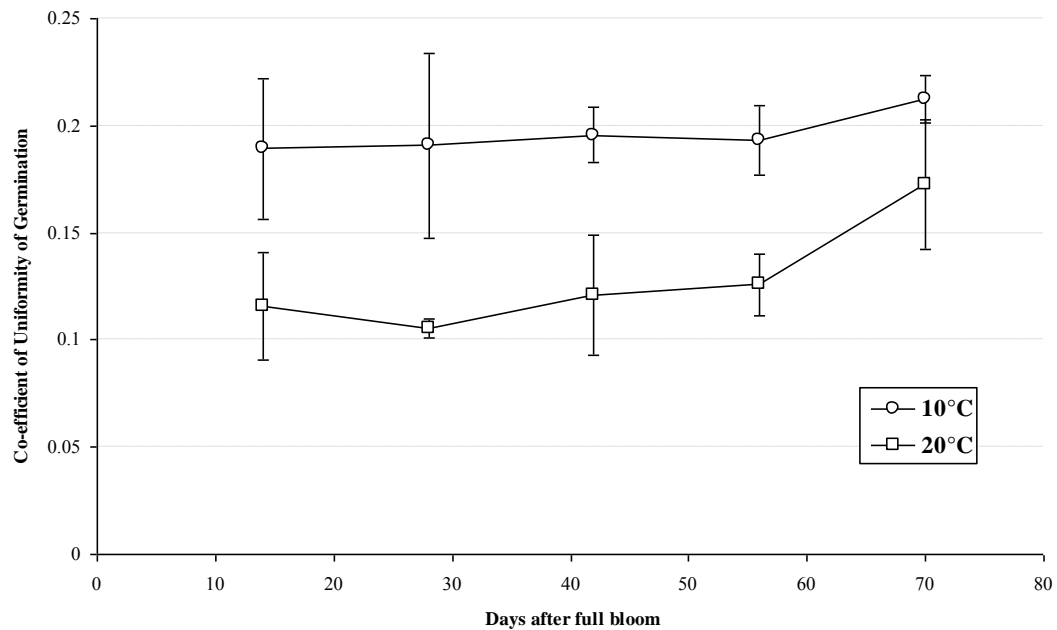


Figure 8: Co-efficient of uniformity of germination of *P. bracteatum* seeds imbibed at 20° and 10°C. Data points are means of 4 replicates of 50 seeds \pm SEM.

SEED GRADING

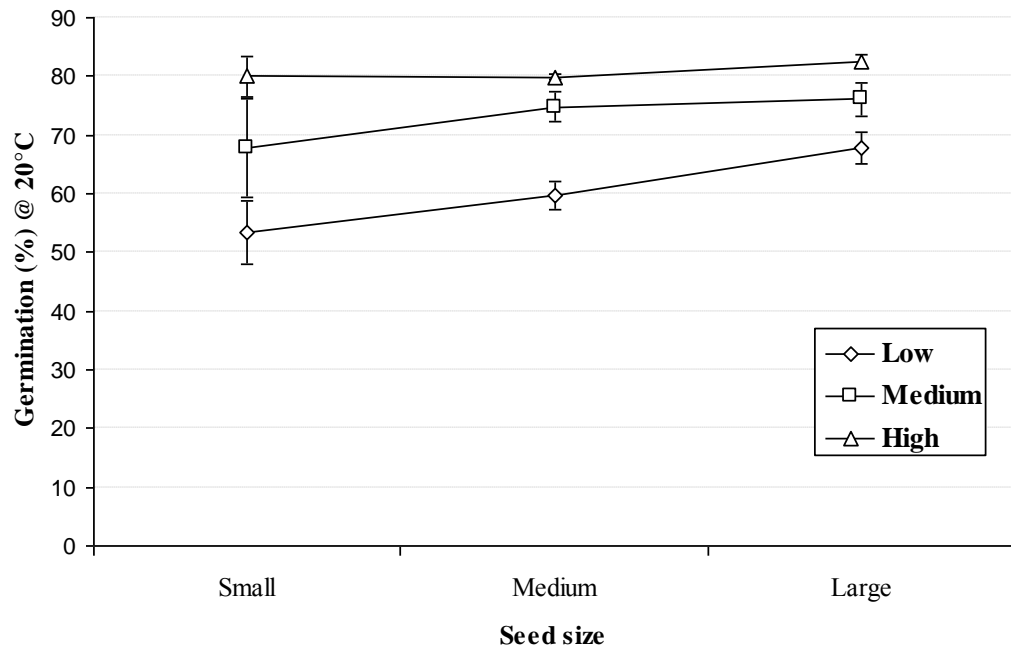
The percentage of *P. bracteatum* seeds that germinated when imbibed at 20°C was significantly effected by seed density ($F_{2,18} = 268.08$; $P < 0.001$) and size ($F_{2,18} = 34.26$; $P < 0.001$) (Figure 9). No significant seed density by size interaction was recorded ($F_{4,18} = 2.64$; $P = 0.068$). Averaged across size class, the level of maximum germination was 60%, 73% and 81% for low, medium and high density seed groupings, respectively (Figure 9). When averaged across density groupings, the level of germination was 67%, 71%, 76% for small, medium and large size classes respectively. A greater proportion of the variation in seed germination was explained by seed density than seed size.

Time to 50% germination (t-50) was significantly effected by grading by seed density ($F_{2,18} = 24.69$; $P < 0.001$), but not by size ($F_{2,18} = 2.58$; $P = 0.104$) (Figure 10), and no significant seed density by seed size ($F_{4,18} = 0.52$; $P = 0.725$) interaction was recorded when seeds were imbibed at 20°C. When averaged across density groupings, the time taken to t-50 germination was higher for small and medium seeds than large seed.

When the seeds where imbibed at 10°C the level of germination was low across all density and size groupings, with maximum germination varying from 18 to 32%. A significant seed density ($F_{2,18} = 105.11$; $P < 0.001$) and size ($F_{2,18} = 17.08$; $P < 0.001$) effect was recorded. No significant interaction between seed density and size on maximum germination ($F_{4,18} = 2.84$; $P = 0.055$) was recorded. Averaged across size class, the level of maximum germination was 19%, 24%, 31% for low, medium and high density seed groupings, respectively (Figure 9). When averaged across density groupings, the level of germination was 21%, 27% and 32% for small, medium and large size classes respectively.

Time to 50% germination (t-50) was also significantly effected by the seed density ($F_{2,18} = 214.46$; $P < 0.001$) and seed size ($F_{2,18} = 15.23$; $P < 0.001$) when imbibed at 10°C. In addition, a Significant interaction between seed density and size on t-50 ($F_{4,18} = 3.70$; $P = 0.023$) was recorded at this temperature. t-50 varied from 8 days for large, high density seed to 9.5 days for small, low density seed. Seed density explained a greater proportion of the variation.

(a)



(b)

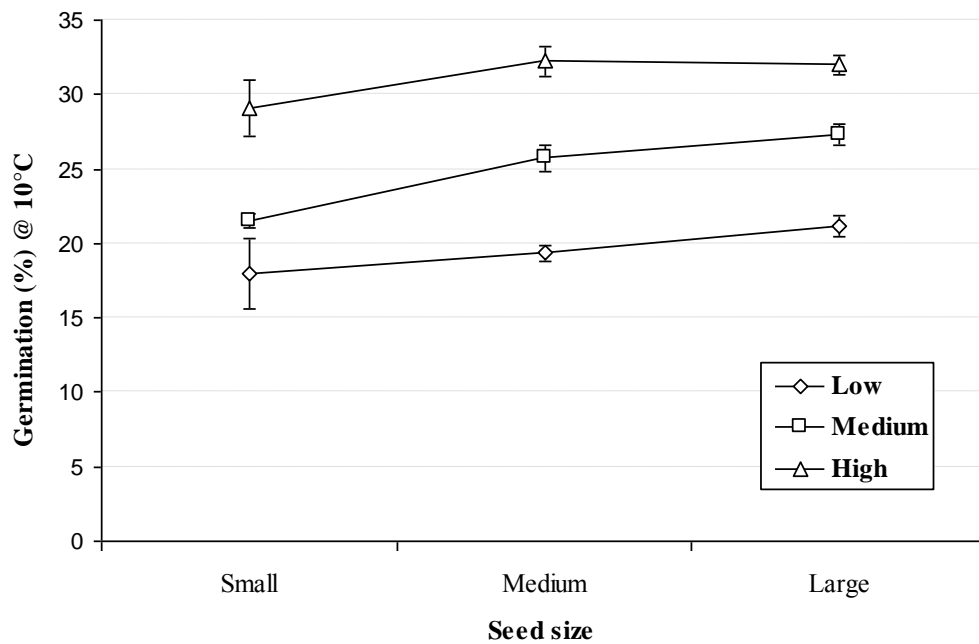
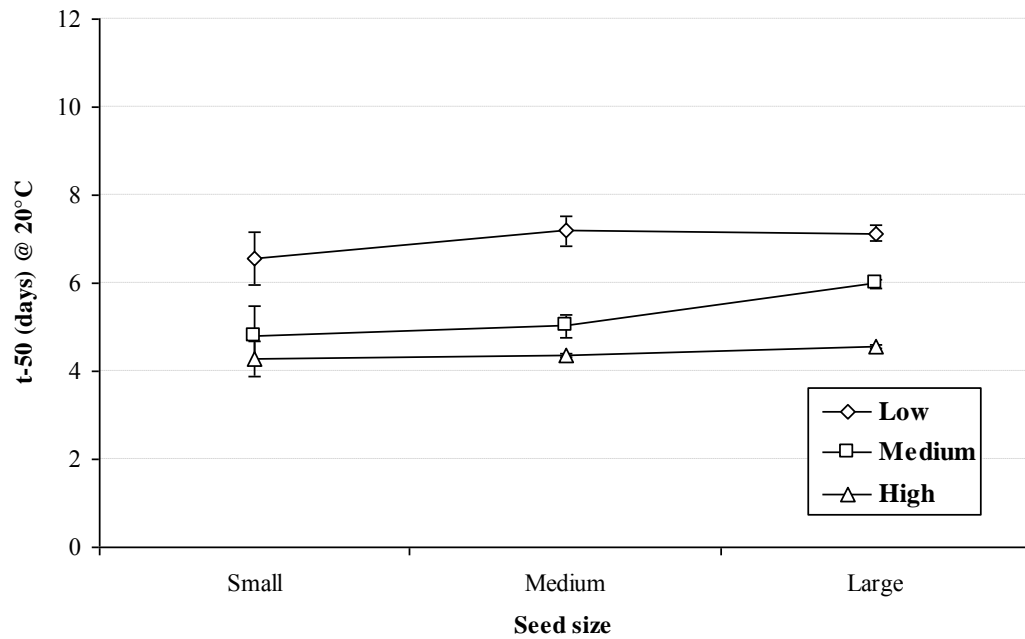


Figure 9: Effect of seed size and density on maximum germination percentage of *P. bracteatum* seeds after 21 days at (a) 20° and (b) 10°C. Data points are means of 3 replicates of 40 seeds \pm SEM.

(a)



(b)

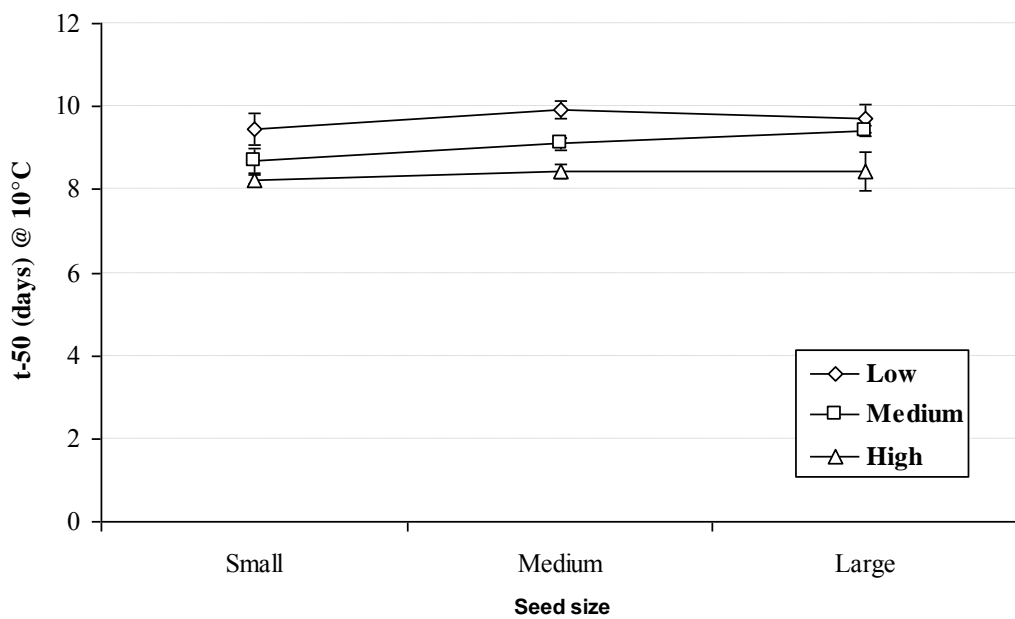


Figure 10: Effect of different sizes and densities on time taken to 50% germination of *P. bracteatum* seeds at (a) 20° and (b) 10°C. Data points are means of 3 replicates of 40 seeds \pm SEM.

DISCUSSION

Commercial production of the previously uncultivated species *Papaver bracteatum* will require development of production guidelines, including recommendations for crop establishment. In Tasmania, commercial production of the related *P. somniferum* crops involves establishment by direct drilling using high quality seed. Similarly, to avoid high costs associated with transplanting seedlings *P. bracteatum* production in Tasmania will require successful crop establishment by direct seeding. For this to be achieved high quality seed is seen as a necessity.

The results from the present study show that seed viability and vigour increased until late in seed development. Germination percentage up to 90% was achieved when seeds were harvested until 70 days after anthesis, at which point capsules were fully desiccated. Pores (Kadereit, 1993) under the capsule cap were first observed to be open, allowing seed dispersal, at approximately 56 DAFB and delaying seed harvest after this time under commercial production conditions would risk loss in seed yield. At 56 DAFB and 70 DAFB no significant difference in germination percentage was observed when imbibed at 20°C but a 20% increase in germination percentage was recorded in the later harvested seed when imbibed at 10°C. The seed weight was significantly higher in seeds harvested at the later date, and this may explain the germination response as larger and heavier seeds displayed greater vigour. While, the reasons for the increase in seed weight late in development were not investigated, it is possible that loss of the lighter seed fraction through pores below the capsule lid may have contributed to the increase in mean seed weight.

Mass maturity in *P. bracteatum* seed was attained at 70 DAFB. This result was in agreement with the other time of harvest studies where later harvest dates provide highest seed quality. High germination percentage (85%) was reported in *Daucus carota* (carrot) when the harvesting was delayed until 70 days after flowering where the normal harvest time was typically between 45 to 55 days (Gray and Steckel, 1983). Delaying the commercial harvest of seed crops under field conditions in cool climates until late in crop maturation is generally recommended due to slower rate of crop development under these climatic conditions. However, delaying harvest time to maximise germination percentage of the seeds must be balanced against potential yield loss.

Complicating the determination of optimum harvest time for *P. bracteatum* seed is the wide range of flower maturities that exist on the plant. As flowering is not synchronous, the several capsules that may be present on any one plant will be at different stages of development at any point in time. Determining optimum harvest maturity at a crop level is therefore predicated upon balancing potential for loss from overmature capsules against low seed quality from immature capsules. Hence the ideal time of harvest should be when as many capsules as possible are mature but before loss of mature seeds from seed shattering or crop lodging (Copeland and McDonald, 1995). Further research at a crop level is required to determine optimum harvest date for *P. bracteatum* seed production, but knowledge of the changes in seed yield and quality at the capsule level with maturity will assist in generating seed production recommendations.

Selection of the most suitable sowing date and adoption of improved cultural practices are often recommended to increase seed quality and yield. Optimum sowing date is an important production consideration (Amanullah *et al.*, 2002) as time of sowing impacts on the growing conditions, and particularly the temperature, under which the seed subsequently develops. Temperature influences the rate of growth and development in all crops (Bonhomme, 2000; Ferreira *et al.*, 1997), and is reflected in the use of degree days calculations for vegetable production and specifically for crops whose life span is dependent on seed quality in the field conditions (Dufault, 1997). Previously the concept of day degrees have been successfully used in many vegetable seed crops, such as corn (Arnold, 1959), cucumber (Perry *et al.*, 1990) and peppers (Perry *et al.*, 1993). The total number of degree days required for a crop to reach maturity is useful for producers to predict the appropriate harvest date to optimize seed quality.

Seed size and density have a significant effect on the germination percentage at 10° and 20°C, with the larger heavier seed fraction providing the highest quality seed. This finding is consistent with studies of many other species. Nerson (2002) reported small seeds of muskmelon germinated poorly and the low seedling growth clearly suggests a possible interaction between physical parameters and seed quality. Vaughton and Ramsey (1997) found that large seeds germinated faster than small seeds in *Krascheninnikovia lanata* (winterfat) and concluded that the response was due to the ability of the large seeds to provide higher energy and nutrients.

The results from these experiments assist in developing recommendations to the commercial poppy industry for seed production. Larger and heavier seeds displayed the greatest germination percentage and vigour and are recommended for sowing where soil temperatures are low. Delaying harvest until capsule pores open is required to achieve adequate seed maturity and therefore seed quality. Delays in harvest after pore opening increase seed quality, but further research under field conditions is required to determine optimum crop harvest maturity since seed loss after pore opening may outweigh gains in seed quality.

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CHAPTER 5

THE PHASE CHANGE IN *PAPAVER BRACTEATUM*

INTRODUCTION

Juvenility, or ripeness to flower, has been defined as the condition of the plant before it is mature enough to flower under normally inductive conditions (Salisbury and Ross, 1985). Higher plants are unable to initiate flowering immediately after germination and have to undergo a process of maturation, or juvenile developmental phase (Martin-Trillo and Martinez-Zapater, 2002). This transition from juvenile to adult characteristics is termed as phase change (Hackett and Murray, 1996; Lavee *et al.*, 1996; Meilan, 1997). This phase of development in which the plant is insensitive to inductive conditions is most common with many seed- raised species (Hedley, 1974; Hedley and Harvey, 1975; Maginnes and Langhans, 1967). Most perennial plants must pass through a significant juvenile phase of vegetative development before they are able to flower (Hopkins, 1999).

The juvenile stage can last from a few days to several weeks or years depending on species or cultivar (Bernier *et al.*, 1981a). The juvenile phase in many woody plants can be very lengthy, with Hackett (1985) reporting a juvenile period of 30-40 years in some forest species while Rugini (1986) and Bellini (1993) reported a juvenile period of greater than 15 years in *Olea europaea* (olives). Brown (1992) reported that juvenile like phase is independent of chronological age but lasted until plants reached a minimum size or stage of development in *Tanacetum cinerariaefolium* L.

(pyrethrum). In *Heuchera* species (Bressingham Hybrids), a juvenility requirement of 10 weeks must be met before satisfying a vernalisation requirement for flowering (Fausey, 2005).

While long lived woody species tend to have lengthy juvenile periods, short lived herbaceous annual and perennial species tend to have a much reduced juvenility requirement. Annual *Papaver* species have been shown to flower in as little as 8 weeks after germination (Wang *et al.*, 1997a), suggesting flower initiation occurred shortly after plant emergence, and was found not to be influenced by temperature. Duration of the juvenile phase was almost unchanged (3-4 days after emergence when transferred from a 16-h to a 9-h photoperiod regime with a thermo period of 25/20°C in *P. somniferum* (Wang *et al.*, 1997b). Collinson *et al.* (1992) reported a significant difference in the duration of juvenile phase in *Oryza sativa* (rice) compared to *P. somniferum*. Cooler temperatures (28/20°C) prolonged the duration of juvenile phase in the four rice cultivars tested in the glasshouse conditions (Collinson *et al.*, 1992). Also, in *Glycine max* (soya bean) another short day plant, the duration of the juvenile phase was also temperature dependent unlike *P. somniferum* (Hodges and French, 1985; Jones and Laing, 1978). No published information exists on the length of the juvenile phase in perennial poppy species, but planting of ornamental perennial poppies is recommended for autumn if flowering is to occur in the following summer (Levy and Palevitch, 1982) so it may be assumed that a juvenile period of several weeks must be met prior to inductive environmental requirements (vernalisation) being met in winter and spring for flowering to occur. Time of planting studies in perennial crops such as pyrethrum (Fulton, 1998) sown from seed demonstrate the need to complete both a juvenile and a flower induction

phase if flowering is to be achieved in the same year. Identification of the length of the juvenile phase is thus important in development of management practices for perennial crops, while an understanding of the physiological basis of juvenility may lead to development of strategies to manipulate the response.

Phase change is a complex process involving environmental, hormonal and genetic factors (Araki, 2001; Bernier, 1986; Evans and Passas, 1994; Kerstetter and Poethig, 1998; Moose and Sisco, 1994; Poethig, 1988). From a research perspective, it is important to document the phase change across species to characterise the traits unique to each phase and thus gain greater understanding of the regulation of phase change (Sylvester *et al.*, 2001). From an applied research perspective, knowledge of the timing of the phase change is important and the identification of morphological traits linked to the transition can assist in scheduling flowering times in the field conditions. Phase change indicators differ between species and may at best be a tool for approximating the timing of the change as they do not measure the underlying changes in gene expression and physiological processes that control the transition.

Many different morphological and physiological changes have been documented to occur at the phase transition in plant species. For many species, plant size appears to be important in the transition to maturity (Klinkhamer *et al.*, 1987) and, in general, conditions that promote growth reduce the duration of the juvenile period (Vince-Prue *et al.*, 1984). The effect of size may be explained by two hypotheses. The first hypothesis is that a plant of sufficient size transmits more than one signal from various plant organs to the meristem, which then undergoes a phase change from juvenile to adult. The second is that the apical meristem behaves independently and

undergoes the phase transition at a time when sufficient meristem size or physiological development has been attained (Vince-Prue *et al.*, 1984). The duration and characteristics of the juvenile and mature phases as well as the two components of the mature phase, the vegetative phase in which the plant is competent to flower but has not received the inductive signal and the reproductive development phase (Thomas and Vince-Prue, 1997), are unique to each plant species. No attempt has been made to establish the duration of these three major phases during the vegetative and reproductive development of *P. bracteatum*.

Progressive changes during the juvenile period may be measured as morphological, anatomical, physiological and developmental differences. Changes include leaf shape, thickness and epidermal characteristics, phyllotaxis, thorniness, shoot orientation, anthocyanin pigmentation, photosynthetic characteristics, disease and insect resistance and competence to form adventitious roots (Hackett and Murray, 1996). These changes differ from species to species and may be dependant on the growing conditions to which the plant is exposed during the juvenile phase (Greenwood *et al.*, 1989; Steele *et al.*, 1989). Hackett (1985) stated that the length of juvenile phase was mainly inherited but could be influenced by environmental factors. Reproductive competence may be accelerated or delayed by varying environmental conditions in herbaceous species. For example, the length of the juvenile phase in *Arabidopsis* was decreased when plants were grown under shorter day length and cooler temperature conditions (Martin-Trillo and Martinez-Zapater, 2002). Where duration of the juvenile phase can vary with growing conditions, expressing the length of the phase in units of time is problematic. Identification of characteristic changes occurring at

the phase transition provides an alternative strategy to assess duration of the juvenile phase.

Leaf number is one of the most widely documented phase transition indicators, and has been used as an indicator of the end of the juvenile phase in a number of plant species. Brewster (1985) proposed leaf number was a stable marker of the end of juvenility in onions grown under different light conditions, whereas time, leaf area and leaf dry weight were not. In these studies, leaf number at phase change was unaffected by varying conditions of irradiance and photoperiod during the juvenile phase. The end of juvenility in *Oryza sativa* (rice) has been characterised by counting either the number of days after sowing or leaf number. Most rice cultivars investigated produced a minimum of five leaves before their juvenile phase was completed (Sasamura, 1960). In *Saccharum officinarum* (Sugar cane) two to three nodes are required for a positive photo-inductive response (Mangelsdorf, 1956). Williams (1960) found that node number was the potential indicator to determine the response to inductive treatment in *Rubus niveus* (Raspberry).

Changes in leaf morphology have been used as phase change indicators in some species. At phase transition, plants of some species display very distinct changes in leaf shape or anatomy, whereas others species show more subtle and gradual transition between juvenile and adult phases (Borchert, 1976; Greenwood, 1995; Hackett, 1985; Sylvester *et al.*, 1990). Poethig (1990) documented that shape of the leaf was one of the possible indicators of vegetative phase change in maize and many legumes with hypogeal germination. *Triticum vulgare* (Wheat) has been considered to be one of the best examples for evaluating phase change because its leaf anatomy

changes as it progresses from a juvenile to adult phase (Kerstetter and Poethig, 1998). In a study of Maize, Bluegrass, and Rice, three distantly related and physiologically distinct grass species, leaf shape rather than leaf surface anatomical features was found to be the most uniform phase change indicator (Sylvester *et al.*, 2001). The presence of leaf abaxial trichomes has been documented as an indicator of the phase change in *Arabidopsis* (Telfer and Poethig, 1998).

While plant size and leaf morphology are the most widely reported indicators on the phase change, other measures have been documented for a smaller range of species. The distance between the apical meristem and the roots was concluded to be the factor that governed phase transition and therefore timing of flower initiation under inductive conditions in *Ribes nigrum* L.(Blackcurrant) and *Nicotiana tabacum* L.(Tobacco) (McDaniel, 1980; Schwabe and Al-Doori, 1973). In *Euphorbia pulcherrima* L.(Poinsettia), a long day photoperiodic species, the time of phase transition was concluded to be a function of the age of the meristem (Evans *et al.*, 1992), while for other herbaceous day length sensitive species, juvenility seems to be due to incompetence in other plant parts, especially the leaves (Lang, 1965). Anatomical traits such as presence or absence of hairs on the leaves have also been linked to the phase transition (Bongard-Pierce *et al.*, 1996; Sylvester *et al.*, 1990).

Given the complexity of the phase transition process, it is perhaps not surprising that this broad range of morphological indicators has been documented. While no indicator applicable to all species has been found, identification of specific indicators for specific agricultural crops and cultivars has been possible and provides a valuable tool in crop research and management. In commercial floriculture, it is very

important to predict the length of the juvenile phase to predict the accuracy of flowering times (Adams et al., 2001) for year round flower production. Knowledge on the length of juvenile phase helps to reduce the cost and time normally required to initiate flowers. Previous studies have reported that by predicting the length of juvenile phase, the timing of inductive treatments can be optimised. For example, in *Oryza sativa* cv. Zuiho (Katayama, 1964) a single inductive cycle and in chrysanthemum a period of eight consecutive short days were required to be sufficient for flower initiation (Cockshull, 1972). Hence the information on the length of juvenile phase is valuable for commercial crops where there is greater potential to manipulate the growing environment and also in producing uniform flowering inturn maximising crop yield.

MATERIALS AND METHODS

PLANT MATERIAL

Genetically uniform *P. bracteatum* seed, collected from a single plant grown under glasshouse conditions, was used in the juvenility experiments. Plants were raised from graded seed germinated in trays filled with a moistened mix of 50% perlite, 25% coarse sand and 25% peat, a potting media found to be ideal for small seeded species (Gracie *et al.*, 2003). The trays were kept in controlled environmental conditions at a daily temperature of $20 \pm 3^{\circ}\text{C}$. The date of emergence was defined as the day when the two cotyledons had unfolded. Seedling emergence occurred approximately 2.5 weeks after sowing. Seedlings were transplanted into 12 cm diameter pots containing potting mix four weeks after sowing, when they could be handled without damage. The potting mix used for seedling growth consisted of peat, sand and pine bark (1:2:7) and had a pH of 6.0. The fertilizer composition in the potting mixture was as follows: slow release (5-6 months) Osmocote® granules (330g/50L), dolomite lime (330g/50L), iron sulphate (25g/50L) and trace elements (Micromax® 20g/50L). The pots were watered daily. Identical fertilizer and irrigation schedules were followed for each of the treatments. Seedlings were grown under glasshouse conditions with ambient light and a temperature of $20 \pm 3^{\circ}\text{C}$ for 12 weeks until the imposition of experimental treatments. One week prior to treatments being imposed, 100 uniform sized plants were re-potted in 16 litre polyethylene pots to ensure plant growth was not restricted by root volume for the duration of the trial. Treatments were imposed 16 weeks after sowing, at which point the plants were 14 weeks old from emergence and had a mean leaf number of 5.2.

TREATMENTS

Two experiments were conducted. The first experiment involved transferring plants at regular intervals from non inductive to inductive (vernalising) conditions for assessment of flowering date, and the second involved comparison of flowering date between plants held under inductive conditions in a glasshouse environment and plants held under inductive conditions in a shadehouse. In both experiments, a number of possible indicators of the phase change were measured.

Temperature in the glasshouse used for non-inductive conditions varied over the duration of the experiment (Table 3). Data loggers were used to measure the air temperature and values were downloaded on to a computer using Gemini Data logger manager software. Mean maximum daytime temperature over the 8 month duration was 29.7°C and a mean minimum temperature over the same duration was 19.8°C. The light levels in glasshouse varied between 600 and 1500 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$. Light intensity was measured using a line quantum sensor (LI-191SA, LI-COR®, Biosciences, USA) attached to a data logger which recorded light intensity every 30 seconds.

Table 3: Mean minimum and maximum temperatures (°C) inside the glasshouse at the Horticultural Research Centre, University of Tasmania, Hobart during the trial conducted from May to December, 2008.

Month	Mean Minimum (°C)	Mean Maximum (°C)
May	11.2	23.9
June	7.6	22.1
July	11.0	21.5
August	10.9	23.4
September	12.8	25.6
October	15.5	27.8
November	18.9	28.5
December	19.8	29.7

Inductive, vernalising conditions were applied to plants using refrigerated growth rooms at $5 \pm 1^\circ\text{C}$. Computer controlled trolley systems transferred plants between a common glasshouse space and the three refrigerated growth rooms on a daily basis. Each of the three trolleys and associated cold chambers had the capacity to hold 15 plants. The trolleys were programmed to move into the glasshouse space at 6 am each morning and return to the adjacent refrigerated chambers at 4 pm in the afternoon. Supplemental lighting was used in the refrigerated chamber to maintain equivalent day length to ambient glasshouse conditions and was provided by combined mercury and fluorescent lights with a photon flux density of $30.2 \mu\text{mol.m}^{-2}.\text{s}^{-1}$. Overhead irrigation was applied up to three times a day, as required based on

plant requirements. Due to space and facility constraints, true replication was not achievable in the experiment design, however continuous monitoring of the chambers confirmed that temperature and photoperiod remained constant in all chambers.

PEST CONTROL MEASURES

To control mite infestations, a predator (*Phytoseiulus persimilis*) was released in an attempt to achieve long term control. Application of Apollo® (a.i. clofentezine) and Calibre® (a. i. hexythiazox) (Miticides) was required when pest population reached a point where damage to plant growth was occurring. This control measure was necessary due to problems in mite control associated with the hairy and dense nature of the foliage of *P. bracteatum*. To prevent white fly (*Encarsia formosa*) infestation, a parasitic nematode biological control agent was released on a regular basis to kill the larvae. The plants were regularly treated with Benlate (Fungaflor), a fungicide to control Fusarium which was a problem in the previous experiments conducted in the glasshouse.

EXPERIMENTAL DESIGN

Random block design of 5 replicates was used in this trial. A total of 100 plants were used for the two experiments, out of which 70 plants were kept in the glasshouse and the remaining 30 plants were kept in the shadehouse. For the main juvenility experiment involving plant transfers, plants were initially held in the glasshouse. Five plants were moved from the non-inductive glasshouse conditions onto trolleys to receive the chilling treatment every 3 weeks. The first transfer was carried out on

April 24th, 2008, and thereafter regular transfers of 5 plants were completed at 21 day intervals. These transfers were continued until the final transfer on September 18th. At the completion of the transfers, 40 plants were held in the inductive conditions and the remaining 30 were used as control plants in non-inductive glasshouse conditions.

Table 4: Temperatures (°C) inside the Shadehouse at the Horticultural Research Centre, University of Tasmania, Hobart during the trial conducted from May to December, 2008.

Month	Mean Minimum (°C)	Mean Maximum (°C)
May	7.0	18.0
June	5.5	16.0
July	4.0	15.0
August	4.5	15.5
September	5.2	20.0
October	7.2	23.0
November	8.0	24.0
December	10.0	24.8

MEASUREMENTS

Plant height, leaf number and leaf length were measured at weekly intervals after planting. Plant height was determined by measuring the distance from the base of the plant to the highest point of the rosette leaf arrangement. As stem elongation is

negligible prior to flower stem development, plant height was essentially a measure of leaf length and the erectness of the leaves in the rosette structure. Leaf number was recorded for each plant, but was not a measure of total number of leaves produced by each plant as some leaf loss due to leaf senescence occurred. Total leaf number was estimated from current leaf number through by addition of an estimate of leaf loss from the rate of leaf senescence. Leaf length of the youngest, fully expanded leaf was assessed using vernier callipers and was a measure of lamina length.

A digital image of the adaxial surface of the youngest fully expanded leaf from each plant was taken each sample date using a Sony cyber-shot (Model DSC TX5P) and used to assess trichome development. As the major visible changes associated with plant ageing were in the distribution of trichomes and an increase in trichome density, a scale of measurement was developed for estimating the hairiness of leaves. Hairiness was assessed visually on a scale of 1 to 5 in order of increasing trichome density (Table 5).

Table 5: Description of the hairiness of the leaves on *P. bracteatum* at various stages of growth

Scale	Description
1	Sparse distribution of hairs. Mostly located around leaf margins
2	Hairs present on lobe of the leaf
3	Greater number of hairs at the lobe
4	Hairs all over the leaf lobe, with low density on the lamina and basal part of the leaf
5	High density of hairs present all over the leaf

Two measures of flowering were recorded; the date at which the flower bud first became visible and the date that the flower bud opened and anthesis occurred. As plants were not observed on a daily basis, the precise date of flower bud appearance was not obtained. Accurate assessment of anthesis was possible as daily assessment were undertaken when each plant approached this stage.

STATISTICAL ANALYSIS

An analysis of variance (ANOVA) was undertaken using Proc GLM in SPSS in accordance with the experimental design. Where treatments were significant, Fishers protected Least Significant Difference (LSD) was calculated to compare treatment means.

RESULTS

Effect of transfer date on flowering:

Significant differences in the time of flowering were found between the transfer date treatments. Flowering was first recorded on July 24th, 27 weeks after plant emergence. None of the plants held in non-vernalising conditions flowered over the 56 week duration of the trial, while all of the plants transferred to the vernalising conditions had reached anthesis in the same time period. The duration between first visible signs of the flower bud and anthesis remained constant between 3 and 4 weeks irrespective of the date of transfer of plants to vernalising conditions.

The shortest duration between transfer to vernalising conditions and anthesis occurred for plants transferred on June 5th and June 26th, indicating that these plants had completed the juvenile phase prior to transfer. Plants transferred on May 15th reached anthesis at a similar date to the subsequent transfer treatment, but received approximately 3 additional weeks exposure to vernalising conditions indicating that the plants completed the juvenile phase while in the vernalising conditions and then received the required duration of vernalisation to induce flowering. The delayed flowering of plants from the first transfer date treatment compared to the two subsequent transfer date treatments may have been due to slower growth rate of the plants in the cooler, vernalising conditions compared to the equivalent juvenile plants maintained under non-vernalising conditions.

Table 6: Date and age of plants at transfer to vernalising (inductive) conditions and mean age when plants reached the visible flower bud and flowering (anthesis) stages. The duration between transfer to inductive conditions and flowering (transfer–anthesis) was calculated from the transfer date and flowering date data for each plant.

Date of transfer	Age when moved to tunnel (weeks)	Mean day of anthesis	Mean, transfer-anthesis (weeks±SE)	Mean age at anthesis (weeks±SE)	Mean date of visible bud	Mean age at visible bud (weeks±SE)
April 24th	14	117.40	15.8±0.95	29.8±0.44	26.40	26.4±0.43
May 15th	17	75.40	10.6±0.64	27.6±0.42	25.20	25.2±0.41
June 5th	20	55.80	8.2±0.53	28.2±0.64	24.80	24.8±0.65
June 26th	23	59.80	8.0±0.62	31.0±0.96	27.40	27.4±1.11
July 17th	26	69.20	9.4±0.63	35.4±1.08	31.80	31.8±1.13
Aug 7th	29	89.40	11.6±1.19	40.6±1.67	37.0	37.0±1.67
Aug 28th	32	115.80	16.4±1.33	48.4±1.56	44.40	44.4±1.56
Sept 18th	35	127.80	17.2±1.71	52.2±0.94	48.20	48.2±0.06

A trend of increasing age at flowering with later transfer dates to vernalising conditions was found from the June 5th transfer treatment onwards, following a small decrease in flowering age between the first and subsequent 2 transfer dates (Figure 11). Given that an 8 week period between commencement of vernalisation and flower opening was the shortest duration found, the data suggest that, under the growing conditions utilised in this experiment, juvenility was completed approximately 20 weeks after emergence.

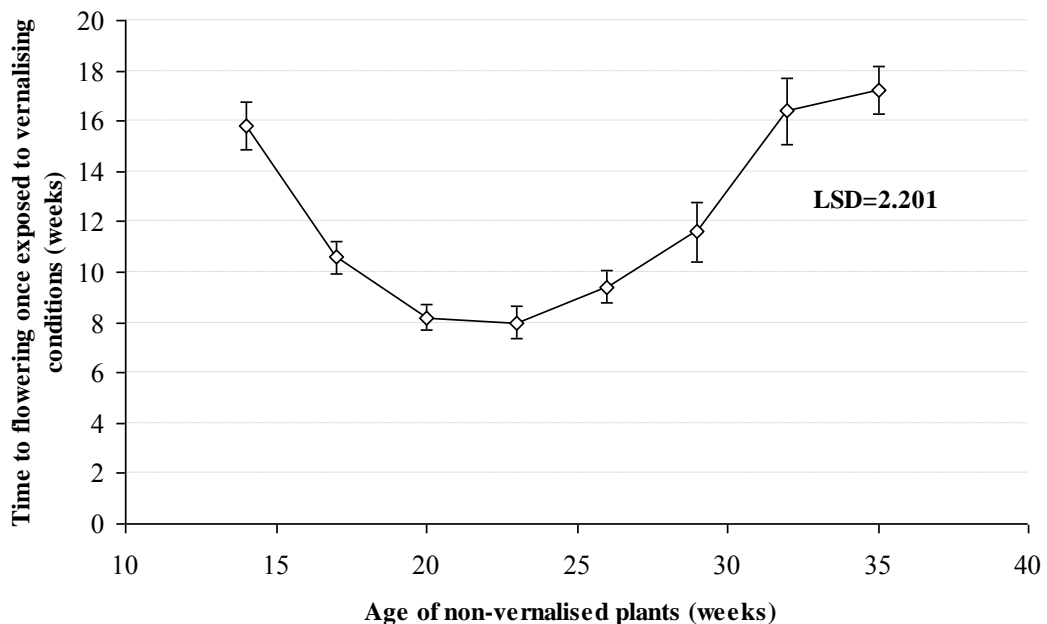


Figure 11: Effect of age of plants at point of transfer from non-vernalising to vernalising conditions on duration in vernalising condition to flowering. Points represent the mean time to flower of five replicates transferred on each occasion for each treatment. Bars represent SE (n=5). LSD (P<0.05) = 2.201.

The increase in plant age at flowering between each of the transfer dates after plants had reached maturity was greater than the 3 week interval between transfer dates. As glasshouse conditions were not constant for the duration of the trial (Table 3), it is probable that changes in conditions affected either the rate of initiation or early development of the flowers.

Variation in flowering date was recorded at each transfer date treatment (Figure 12), with replicate plants generally flowering within a 4 week window for all treatments apart from the final 2 transfer dates where one plant in each treatment displayed delayed flowering. Despite the variability in flowering response, analysis of flowering age data revealed significant differences in flowering age between treatments. The trend of increasing age at flowering with sequential transfer dates was seen for transfers after the fourth treatment (June 26th, 23 weeks), with a slope greater than 1 (Figure 12) highlighting the delay in responding to the inductive conditions after juvenility had been completed.

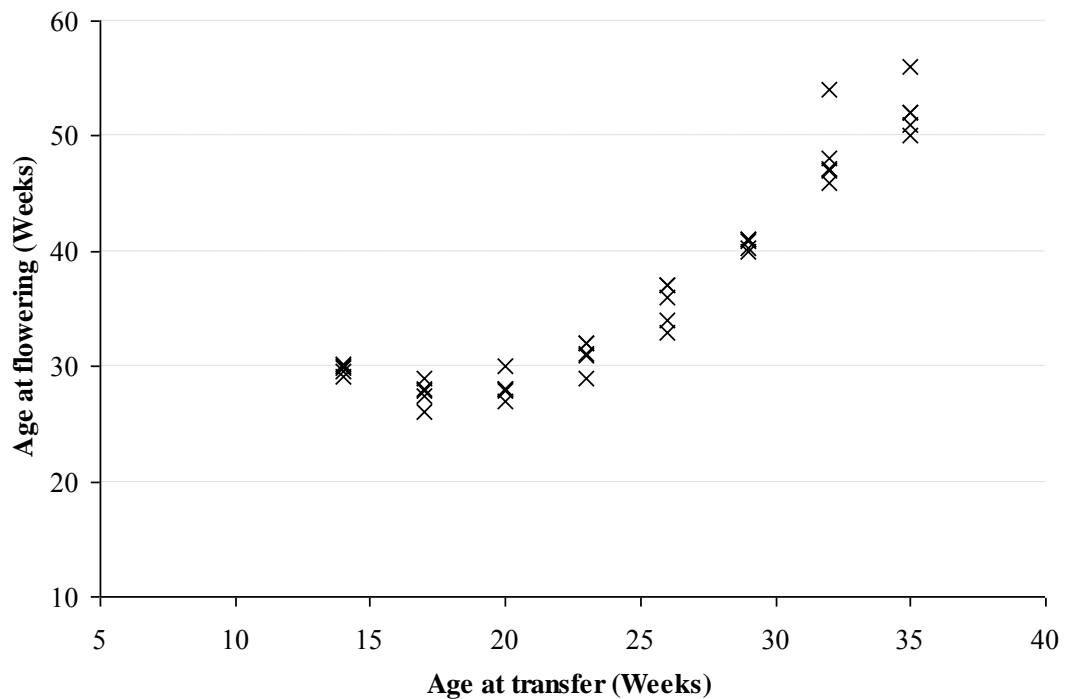


Figure 12: Age at flowering for individual plant transferred from non-inductive to inductive conditions at intervals of three weeks. Points represent the date of flowering for each plant.

Leaf number per plant:

Fully expanded leaf number increased at a rate of approximately 1.6 per week between weeks 16 and 28, with a similar rate of leaf initiation found regardless of whether plants were held in inductive or non-inductive conditions (Figure 13). On the basis that the phase change from juvenile to mature plants occurred at week 20, a leaf number of approximately 16 corresponded to this change. Leaf number in 20 week old plants varied from 15.4 in non vernalised plants, 15.8 in plants transferred to vernalising conditions at 14 weeks old (treatment 1) and 17 in plants transferred to vernalising conditions at 17 weeks old (treatment 2), with the differences between leaf number not being statistically significant. Leaf number increased rapidly from week 28 in treatment 2 and treatment 3 plants, with a slower increase noted for treatments 1 and 4 as well as non-vernalised plants. The increase in leaf number in treatments 2 and 3, and to a lesser extent treatment 3, occurred at the time when flower buds had been initiated and were developing on the plant.

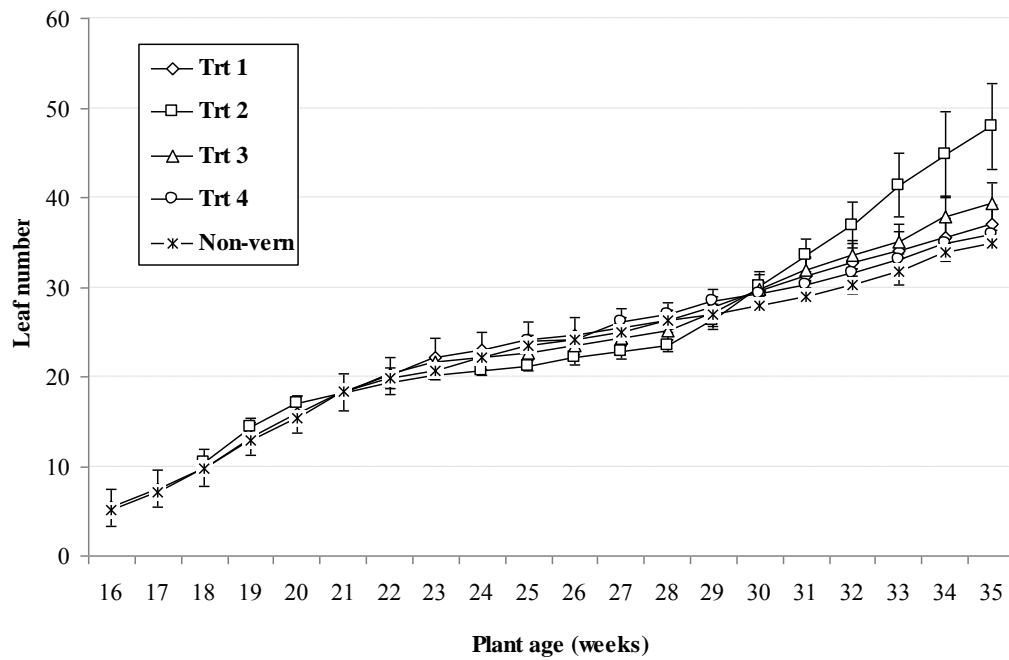


Figure 13: Changes in total number of leaves produced over time by plants held in non-vernalising (Non-vern) conditions or transferred to vernalising conditions when 14 (Trt1), 17 (Trt 2) 20 (Trt 3) and 23 (Trt 4) weeks old. Points represent the mean leaf numbers of five replicate plants. Bars represent SEM (n=5).

Lamina length of the youngest fully expanded leaf increased in all treatments over the duration of the experiment, but in non-vernalised plants the rate of increase slowed from approximately 23 weeks onwards (Figure 14). A large increase in leaf length was noted in treatments 2 and 3 within a week of transfer of plants to vernalising conditions. Leaf length in 20 week old plants varied from 24.46 cm in non-vernalised plants to 24.12 cm in treatment 1 plants and 29.17 in treatment 2 plants.

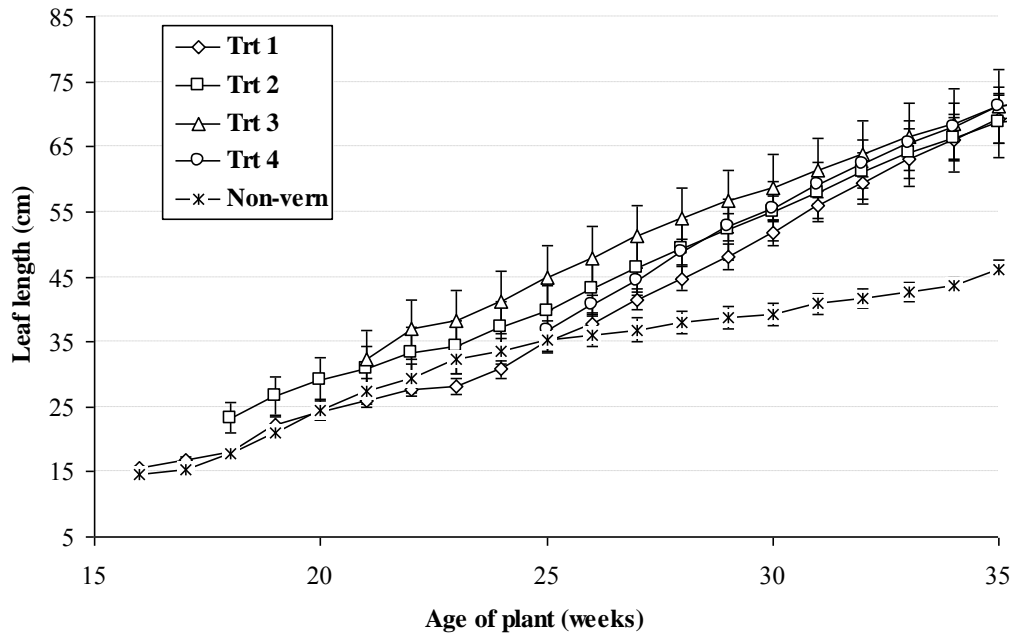


Figure 14: The effect of various treatments on the leaf length. Points represent the mean time to increase in length of the leaves of five replicates transferred on each occasion. Bars represent SEM (n=5).

The trend in plant height was similar to that of leaf length, with an increase over time and a slower rate of increase in non-vernalised plants (Figure 14). The increase in plant height following exposure to vernalising conditions is likely to have resulted from a combination of the increased leaf length noted above and an increased erectness of leaves observed following vernalisation.

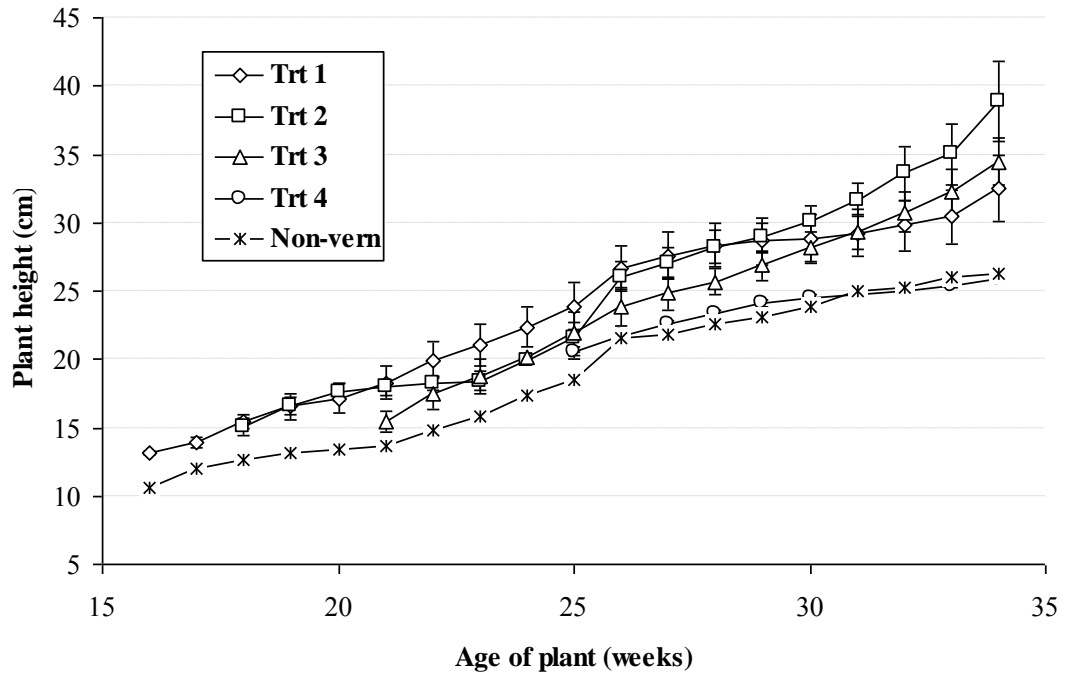


Figure 15: Effect of transfer date treatments on plant height. Points represent the mean of five replicates transferred on each occasion. Bars represent SEM (n=5).

An increase in leaf hairiness with plant age was noted (Table 7). Plants less than 26 weeks old scored 1 or 2 for the leaf hairiness to describe trichome density and distribution on the youngest, fully expanded leaf of each plant. Leaf hairiness was only assessed at the time of transfer to vernalising conditions and at anthesis, so differences in trichome development under the various transfer date treatments could not be assessed.

Table 7: Density and distribution of trichomes on the upper side of the leaf of plants transferred at different times and at the point of flowering.

Treatment	Age at transfer	Mean hairiness at transfer	Mean hairiness at anthesis
1	14	1.2	1.8
2	17	1.0	1.4
3	20	1.2	1.6
4	23	1.8	2.6
5	26	1.8	2.8
6	29	2.4	3.6
7	32	2.6	4.0
8	35	2.6	4.4

Hairiness was assessed using from digital images of leaf surfaces and using the scale described in the methods section (Table 5). Hairiness score are means of five replicates.

A comparison between flowering times and plant morphology for plants held under the shadehouse conditions and both vernalising and non-vernalising conditions in the glasshouse. Flowering occurred September 21st and October 10th in plants held under shadehouse conditions, compared to July 16th to August 4th for plants held under vernalising conditions in the glasshouse from 14 weeks of age. Plants held in non-inductive conditions in the glasshouse did not flower. Growth rate under shadehouse conditions was slower than under glasshouse conditions, and this difference was evident in the slower rate of leaf production (Figure 16). The rate of leaf production under shadehouse conditions increased from plant age of 35 weeks, corresponding to the increase in temperature in the shadehouse in the late August/September period (Table 4) that corresponded to this plant age.

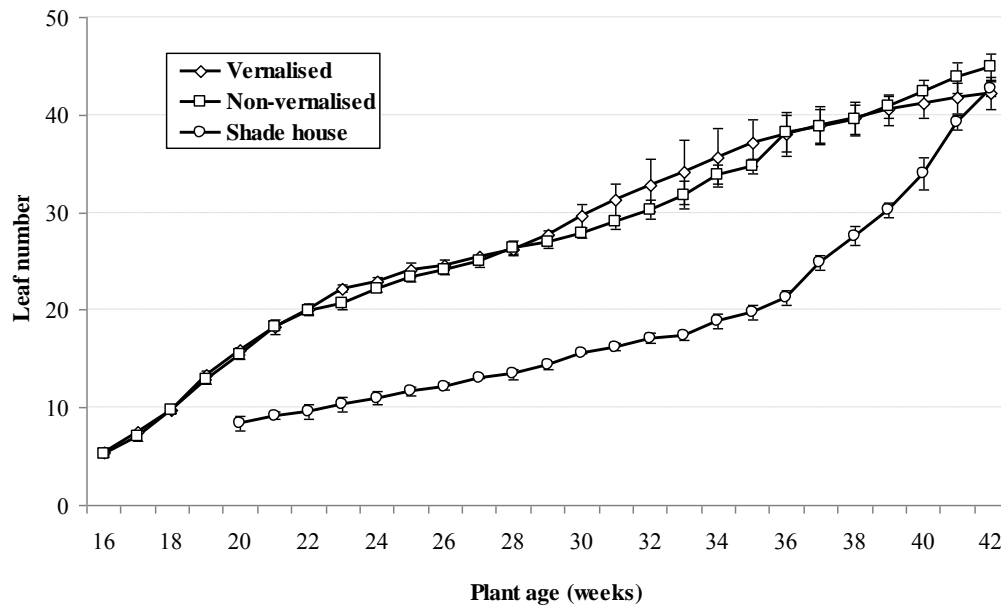


Figure 16: Effect of leaf number in shadehouse and in Non-vernalised conditions (on the bench inside the glasshouse) and in glasshouse (vernalised conditions) at different dates. Points represent the mean of five replicates. Bars represent SEM (n=5).

The period between flower buds first being visible and anthesis was between 3 and 4 weeks in shadehouse grown plants. As this timeframe corresponded to that noted in glasshouse grown plants, it was assumed that the duration between initiation of flowering and anthesis would be similar under both conditions. As flower initiation was found to occur 8 weeks prior to anthesis, an analysis of potential markers of the phase change in *P. bracteatum* at 8 weeks prior to anthesis under shadehouse and glasshouse conditions was undertaken (Table 8).

Table 8: Mean of leaf number, leaf length, plant height, leaf hairiness at eight weeks prior to flowering for each treatment.

Date of Transfer	Leaf Number(\pm SE)	Leaf length(\pm SE)	Plant height (\pm SE)	Mean leaf hairiness at flowering
April 24th	18.3 \pm 0.3	25.96 \pm 1.05	19.96 \pm 1.37	1.8
May 15th	17.0 \pm 0.4	29.17 \pm 3.27	17.56 \pm 0.64	1.4
June 5th	17.1 \pm 0.5	29.34 \pm 4.06	16.87 \pm 0.84	1.6
June 26th	20.6 \pm 0.6	36.32 \pm 1.45	20.43 \pm 0.29	2.6
July 17th	24.1 \pm 0.7	47.05 \pm 1.38	22.68 \pm 0.58	2.8
August 7th	27.0 \pm 0.8	59.93 \pm 1.73	28.40 \pm 1.43	3.6
August 28th	30.3 \pm 2.6	54.29 \pm 0.84	28.91 \pm 1.20	4.0
September 18th	34.8 \pm 2.4	54.78 \pm 1.61	25.76 \pm 0.63	4.4
Shadehouse	17.1 \pm 0.6	60.05 \pm 1.52	25.64 \pm 0.29	3.6

Under both glasshouse and shadehouse conditions, plants exposed to inductive conditions while still juvenile or just at the point of phase transition had between 17 and 18.3 fully expanded leaves. Plants transferred to inductive conditions when mature (transfer dates after June 5th) had a higher number of leaves at the point of initiation, reflecting the production of leaves under non-inductive conditions after the phase change had occurred. Leaf length, plant height and leaf hairiness were not useful indicators of the phase change transition as large differences were noted between plants held under shadehouse and glasshouse conditions.

DISCUSSION

An extended juvenile phase of development, lasting approximately 5 months under glasshouse conditions and longer under shadehouse conditions, was demonstrated in *P. bracteatum*. The phase change from juvenile to mature growth was related to plants achieving a minimum size or stage of development, but was not linked to the age of the plant as varying growth rate by holding plants under different environments resulted in differences in the plant age at which the phase change occurred. This finding is in agreement with the previously published data on the juvenile phase of several woody and herbaceous species (Robinson and Wareing, 1969). While further experiments utilising different controlled environment and field conditions, and a range of *P. bracteatum* germplasm, is recommended to confirm the extended duration of the juvenile phase, it was concluded on the basis of the current results that a Spring field sowing would be unlikely to lead to flowering in the first Summer season after sowing so early Autumn planting is recommended for commercial production.

The requirement for a period of vernalisation was evident in the flowering responses measured in the experiments described in this chapter. Plants held under glasshouse conditions did not flower. Mean minimum temperatures in the glasshouse at the point where plants completed the juvenile phase were below 8 degrees and between 10 and 12 degrees for the following 2 months, suggesting a vernalisation requirement at a lower temperature or for extended periods each night as was imposed in the inductive treatment and experienced in the shadehouse conditions. The increase in time taken from imposition of vernalisation to anthesis with increasing plant age after the phase

change was unexpected, and indicated that factors other than vernalisation were involved in the flower initiation and/or development processes. Exposure to high day time temperatures during vernalisation has been shown to delay or prevent flowering in other species (Schwabe, 1955) and has been referred to as de-vernalisation (Bernier *et al.*, 1981a). It is possible that this response also occurs in *P. bracteatum*, and may have implication for field production of the crop in warmer climatic zones.

The end of juvenility was defined by the initiation of a critical number of leaves, with 17 mature, fully expanded leaves concluded to be a useful indicator of the phase change. Leaf number was previously reported as an indicator of phase change in some cultivars of Brassica (Sadik, 1967) while leaf number measured indirectly as number of nodes produced was identified as an indicator of phase change in tobacco (Singer and McDaniel, 1986). Previous studies by Bradley *et al.* (1997) and Adams *et al.* (1998) reported that plants which develop a terminal inflorescence, the leaf number below the flower is can be used in predicting the timing of flower initiation. *P. bracteatum* plants contained both terminal flower stems and lateral vegetative rosette shoots at flowering, with leaf number continuing to increase during flowering through growth of the lateral shoots. Further studies under a wider range of conditions, and utilizing a range of germplasm, are recommended to confirm the applicability of leaf number as an indicator of the phase change in *P. bracteatum*.

Leaf morphological characteristics used as phase change indicators in other species were shown not to be applicable in *P. bracteatum*. Trichome distribution and density (Kolodziejek *et al.*, 2006) leaf size (Kerstetter and Poethig, 1998) and plant height, which were a measure of leaf erectness and leaf length, varied considerably with

growing conditions and did not provide a consistent value at the phase change that could be used as an indicator. The slow rate of change in hairiness during the early stages of the experiment suggest that leaf hairiness, if assessed using the scale developed in this experiment, is unlikely to be a sensitive indicator of the phase transition in *P. bracteatum*.

CHAPTER 6

FLOWERING IN *PAPAVER BRACTEATUM*

INTRODUCTION

Characterisation of the flowering response of *P. bracteatum* was one of the aims of the project, and a series of experiments focussed on plant response to vernalisation were conducted over a three year period. *P. bracteatum* is essentially an uncultivated species with limited selection of germplasm available for experimental assessment of flowering behaviour. During the course of the project, several difficulties in culturing of the available plant material in glasshouse conditions were encountered. These included mite infestations that proved difficult to control due to the hirsute nature of the leaves, pathogens damaging leaves and flower buds, and a plant dormancy response whereby cessation of growth and senescence of leaves was observed for a period of approximately 2 months before new growth commenced. These problems in plant culture were most severe in mature plants. Identification of these issues during early experiments in the project forced a shift in emphasis in experiments, with focus shifting to development of plant management strategies to permit study of flowering and assessment of specific aspects of flower development. Five experiments, yielding data on plant cultural practice and flowering response, were completed in the project.

There is currently a lack of scientific knowledge on the flowering of *P. bracteatum* despite the fact that the crops economic success depends on the yield of flowers.

Based on the cold winter conditions experienced in its centre of origin and documented spring time flowering pattern in its natural habitat (Levy *et al.*, 1986), a vernalisation requirement for flower initiation may be expected if any environmental requirements for flowering are present in this species. A day length requirement may also be present given the related annual species *P. somniferum* displays a day length sensitive flowering response (Wang *et al.*, 1997b).

The low temperature requirement for flower initiation is most common in perennial rosette plants, grasses and many of the winter cereals (Brown, 1992). Sung and Amasino (2004) defined this low temperature requirement as vernalisation, where flowering is facilitated by a prolonged exposure to cold treatments. The vernalisation stimulus is perceived by the apex and is not translocated within the plant (Napp-Zinn, 1987), with the transmission of vernalised conditions occurring through mitotic division of cells which have been exposed to cold treatment to induce the vernalised state. Bernier *et al.* (1981a) stated that plant material is only sensitive to cold if it contains actively dividing cells in its apical meristem. The key genes involved in the vernalisation pathway have been identified, and links between the vernalisation and day length sensitive flowering pathways have been characterised in the model crop *Arabidopsis* (Putterill *et al.*, 2004). A dual requirement of short days and low temperature to initiate flowering has been reported in species including many temperate grasses (Heide, 1990) and a combined day length and vernalisation requirement for flowering in *P. bracteatum* cannot be ruled out.

The metabolic pathways involved in flower initiation and development are driven by the changes in gene expression associated with detection of environmental stimuli.

Plant hormones or growth regulators are involved in the metabolic pathways. Application of the growth hormone gibberellic acid was reported to substitute for the vernalisation requirement in many perennial species (Cockshull, 1985). Gibberellins promote stem elongation and flowering in many plants (Pharis and King, 1985; Phinney, 1985; Zeevaart, 1983) and have been widely reported to have a role in the regulation of events following vernalization (Chailakhyan and Lozhnikova, 1962; Hazebroek and Metzger, 1990; Lang, 1957; Lang, 1965; Metzger, 1990). In long-day and biennial plants, gibberellins have been shown to promote flower initiation in non-inductive conditions (King *et al.*, 2001; Lang, 1957; Pharis and King, 1985; Zeevaart, 1983). Previous studies on the effect of GA₃ on flowering in *P. bracteatum* found enhanced flowering and higher thebaine yield (Levy, 1985). Flowering occurred in untreated plants in the study, and it was not clear if the effect of the gibberellic acid application was through partial substitution of a vernalisation requirement or through stimulation of flower development following initiation. The response does, however, provide further evidence that a vernalisation requirement for flower initiation is likely to exist in *P. bracteatum*.

Variations in temperature and light intensity following flower initiation have been shown to affect subsequent flower development. In cold requiring species, exposure to high temperatures has an opposite effect to low temperature vernalisation, and the response has been termed de-vernalisation (Bernier *et al.*, 1981a). Weibe *et al.* (1992) described de-vernalisation as the inability of the plants to attain the reproductive phase after an extended period of cold treatment which is normally sufficient for floral initiation. Halevy (1985) proposed that de-vernalisation is a process during which the vernalised state is partially or completely reversed due to high

temperatures. Michaels and Amasino (2000) reported that the timing of exposure to low and high temperatures was critical to the response, with de-vernalisation ineffective if plants were exposed to high temperatures after a delay of several days post-vernalisation. To date, no examination of a possible de-vernalisation mechanism has been undertaken in *P. bracteatum*.

In addition to high temperature effects following vernalisation, light intensity may influence flowering time. Plants grown under low light conditions may display accelerated flowering and this effect is often ascribed to stress associated with low light levels (Cerdeira and Chory, 2003; Pierik *et al.*, 2004). The sensitivity of *P. bracteatum* flower development to varying light and temperature conditions following flower initiation has not been investigated, but need to be considered in flowering studies as the patterns of flower development noted after imposing treatments to examine initiation may be significantly modified by the post initiation environment.

The evidence from the literature suggests that *P. bracteatum* is likely to have a vernalisation requirement for flower initiation, and the series of experiments undertaken in the project to examine the flowering response therefore focussed on vernalisation.

MATERIALS AND METHODS

Five separate experiments examining aspects of flowering and plant development were completed during the project and a further four were terminated due to poor growth of plants. The experiments involved seed raised plants grown in pots and were conducted in the Shadehouses, glasshouses and growth chambers at the Horticultural Research Centre, University of Tasmania. Also, the variations between the plants were minimal before imposing the treatments to a limited number of population.

EXPERIMENT 1: EFFECT OF VERNALISATION ON FLOWERING IN *P. BRACTEATUM*

An initial trial was undertaken at the commencement of the project using 18 week old plants held under shadehouse conditions. Plant number was limited as the plants were part of a small population grown from original seed stock imported from Israel and intended for seed production. Eight 18 weeks old seedlings were transferred into 16 litre pots and used for the trial which was conducted over a 16 week period between September, 2006 and December, 2006. Four of the plants were kept in vernalising conditions (cold treatment in the controlled environment cabinets) and were compared with the remaining four plants in non vernalising conditions (glasshouse conditions). Data loggers were used to measure the air temperature and values were downloaded on to a computer using Gemini Data logger manager software. Non vernalising glasshouse conditions consisted of the mean maximum daytime temperatures of 29.7°C and minimum temperature of 19.8°C. Vernalising conditions consisted of the mean maximum daytime temperatures of 29.7°C and a night temperature of 5°C. A 10 hour day and 14 hour night length was maintained in

the vernalising treatment, with the day period commencing at 6:00 am each morning and plants returned to controlled temperature chambers at 4 pm in the afternoon. To control mite infestations, a predator (*Phytoseiulus persimilis*) was released in an attempt to achieve long term control. Application of Apollo, Betamite (Miticides) was applied when pest population reached a point where damage to plant growth was persistent.

EXPERIMENT 2: EFFECT OF SHADING ON GROWTH OF *P. BRACTEATUM*

Following the completion of the first experiment, it was noted that the plants rapidly senesced and remained in a dormant state for approximately 2 months before re-sprouting. A second experiment, repeating the first but with greater replication, was terminated as many of the plants progressed to a dormant state before flowering. The leaf senescence symptoms preceding dormancy which were characterised by necrotic and chlorotic regions on exposed leaf surfaces were consistent with photo-damage. It was hypothesised that exposure to high light intensity during periods of elevated temperature such as those experienced in the glasshouse in the summer months may cause sufficient leaf damage to induce a dormant state in *P. bracteatum*. An experiment was therefore undertaken to document the response of plants to varying light intensities.

Sixteen mature plants in 16L planter bags were used for this experiment and four levels of shade were tested. Plants were grown in the glasshouse at the Horticultural Research Centre, University of Tasmania. Temperatures in the glasshouses were maintained at day/night temperatures of approximately 25/15°C. Plants were irrigated

using a capillary mat system (Aquamat S10, Soleno Textiles, Quebec, Canada) with water applied through the matting every 8 hours.

Light intensity was manipulated by placing shade cloth tents over individual plants. Wooden pegs were inserted into the plastic bags to hold the shade cloth above the plant. Different levels of shade were achieved by using the shade cloth of different thickness. The four levels of shading used in this experiment were as follows:

- Level 1: No Shade (Control treatment)
- Level 2: Low Shade (25 % light exclusion by shade cloth).
- Level 3: Moderate Shade (50% light exclusion by shade cloth).
- Level 4: Heavy shade (75 % light exclusion by shade cloth).

Plants were grown under the four light intensity treatments for a period of 20 weeks from May, 2007 to September, 2007. Maximum daily light levels outside the glasshouse varied between 1200 to 2000 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ over the course of the experiment (Figure 17). Assessment of light levels inside and outside the glasshouse revealed a 12% reduction in light intensity due to the glasshouse structure. Plants were assessed on a weekly basis, with leaf number, leaf length and plant height measured for each plant. Leaf number and plant height were assessed as described previously. Leaf length of the youngest, fully expanded leaf was assessed using vernier callipers and was a measure of lamina length. General appearance and health of plants was also noted.

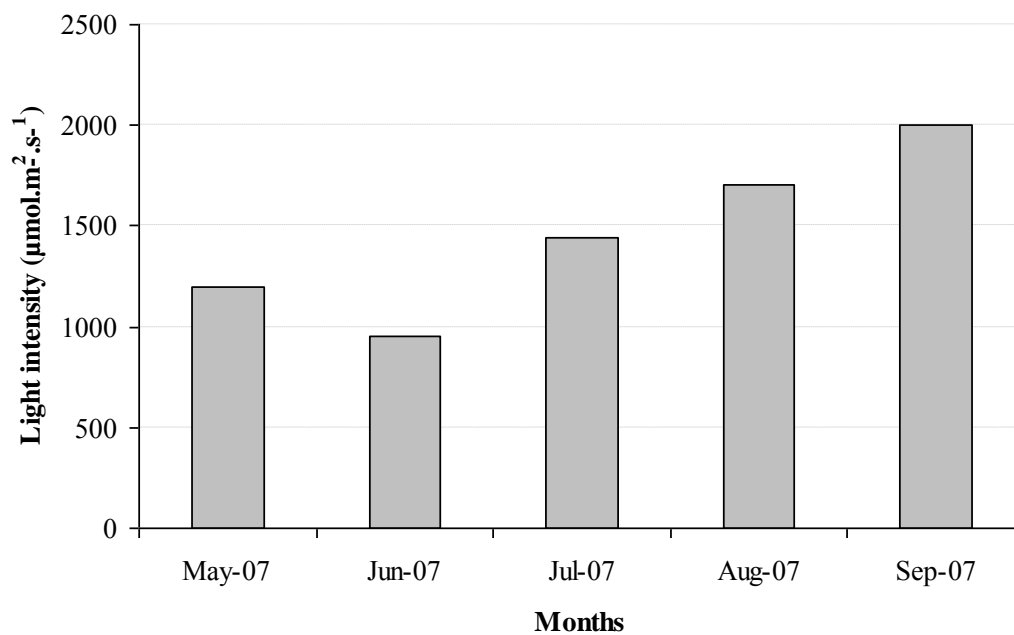


Figure 17: Mean light intensity levels outside the glasshouse at the Horticultural Research Centre, University of Tasmania, Hobart during the trial conducted from May to September, 2007.

EXPERIMENT 3: EFFECT OF SHADING AND TEMPERATURE ON GROWTH OF *P. BRACTEATUM* DURING SUMMER

A second observational experiment examining the effects of shading and temperature was undertaken over the summer period, from December, 2008 to the end of April, 2009. Forty five mature, 48 week old, plants were used in the experiment. Plants had been maintained under glasshouse conditions without shading prior to the commencement of the experiment. Fifteen plants were placed under 50% shade cloth inside the glasshouse (Plate 3), fifteen plants were left unshaded inside the glasshouse and the remaining fifteen plants were kept under ambient light and temperature conditions outside the glasshouse. Maximum daily light levels outside the glasshouse varied between 1200 to 2000 $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ over the course of the experiment. Weekly measurements of plant height, leaf number, were taken to observe plant growth rate under the two different conditions.



Plate 3: Plants kept under the shade cloth and the irrigation was provided through capillary mat system.

EXPERIMENT 4: EFFECT OF GIBBERELLIC ACID APPLICATION ON FLOWERING OF *P. BRACTEATUM*

The slow growth rate of plants grown under glasshouse conditions during the summer months and propensity for plants to become dormant during periods of high temperature and high light intensity created difficulties in conducting vernalisation experiments using the refrigerated growth rooms attached to the glasshouse. Healthy plant growth under shadehouse conditions during summer was possible. An experiment examining the effects of application of gibberellic acid and a gibberellin synthesis inhibitor on flowering of *P. bracteatum* was therefore undertaken to gain indirect evidence of a vernalisation requirement for flower initiation.

The experiment was carried out between November and December and used thirty mature, 40 week old plants in six litre pots. Plants were held under glasshouse conditions prior to commencement of the experiment. Ten plants were used in each of three treatments. In the first treatment, plants were treated with 250 mg/l of gibberellic acid (GA₃ 90% purity, SIGMA-ALDRICH, Australia), sprayed twice with a 10 day interval between applications. In the second treatment, ten plants were treated with a gibberellin synthesis inhibitor, Paclobutrazol (trade name Cultar® available as a Wettable powder with 250g active ingredient per litre, CROP CARE, Australasia). In the third treatment, ten plants were sprayed with tap water. In each treatment, spray was applied to the point of insipient run off. The average maximal and minimal temperatures during the period of the experiment were 20°C and 10°C respectively. Plants were observed regularly and the date of (flowering) recorded for each plant. Plant height and leaf number were recorded on a weekly basis.

EXPERIMENT 5: SHOOT APICAL MERISTEM MORPHOLOGICAL CHANGES DURING FLOWERING

The first sign of flower initiation visible on intact plants is the emergence of the flower bud from the rosette leaf arrangement. This occurs at least 4 weeks after plants are exposed to inductive conditions, and after many of the early events of flower development have occurred. Changes in meristem appearance are amongst the earliest anatomical changes after flower initiation in plants (Sylvester *et al.*, 2001), and identification of early changes can be used in studies of flower initiation. As there is some evidence that the rate of early flower development processes in *P. bracteatum* may be influenced by conditions after initiation (Chapter 5), the ability to identify when initiation has occurred is important in order to separate the effects of treatments on initiation and subsequent flower development. Experimental work was therefore undertaken to document changes in apical morphology during flowering in *P. bracteatum* and to define key morphological stages that could be used in future studies of flowering in the species.

Meristem samples were taken from plants grown under a wide range of environmental conditions and experimental treatments. Vegetative and floral buds were dissected under a Nikon stereoscopic microscope with a 6.3X zoom ratio. Repeated dissections of many apical meristems allowed for the identification of stages of floral development. This analysis was used to generate a series of descriptions of key stages in meristem development.

Several fresh specimens were examined using an environmental scanning electron microscope (FEI Quanta 600, ESEM) in order to record detailed images of meristem

anatomy at different developmental stages. Scanning electron microscopy was performed at the Central Science Laboratory of University of Tasmania. The instrument was operated in variable pressure mode at a water vapour pressure of 1 torque. The samples were cooled to a temperature of 1°C in the SEM chamber using a Peltier stage to slow down dehydration. Further parameter settings such as accelerating voltage (HV), working distance (WD), and magnification (Mag) are shown under the images presented in the results section. Meristem tissue which had reached an advanced reproductive stage was larger than was practicable for use in the ESEM and therefore these stages of development were recorded using a Zeiss Tessoar (parafoal zoom lens optimised for close ups and magnification ranged from 0.4x – 12.8x).

RESULTS

EXPERIMENT 1: EFFECT OF VERNALISATION ON FLOWERING IN *P. BRACTEATUM*

All plants exposed to vernalising conditions flowered and reached anthesis on average 11.5 weeks after being moved to the vernalising conditions, while none of the plants maintained in non-vernalising conditions flowered. The ages of the four vernalised plants at anthesis were 29, 30, 30, and 29 weeks.

Exposure to vernalising conditions resulted in an increase in the height of plants and number of leaves (Table 9). Plant height, measured from the base of the plant to the uppermost leaf tip, increased in vernalised plants after the flower buds became visible on the plants. The increase in leaf number may have been a result of an increase in the rate of leaf initiation, a decrease in the rate of leaf senescence, or a combination of both processes. Reduced plant vigour was observed in the non-vernalised plants and leaf chlorosis was evident in many leaves at the end of the experiment. Leaf chlorosis was also evident in vernalised plants but to a lesser degree than the non-vernalised plants. In both cases, accelerated leaf senescence and plant dormancy occurred within 2 months of completion of the experiment.

Table 9: Mean (\pm S.E.) Plant height (cm) and number of leaves produced before and after inductive conditions over a period of 16 weeks. The mean age of the plants at the time of flowering in vernalising conditions was 29.5 ± 0.29 .

Plant Age (Weeks)	Vernalised conditions Plant Height (Mean \pm S.E)	Non-Vernalised conditions Plant Height (Mean \pm S.E)
20	13.25 \pm 0.17	13.20 \pm 0.23
22	13.62 \pm 0.14	13.52 \pm 0.16
24	14.10 \pm 0.14	13.80 \pm 0.15
26	14.72 \pm 0.19	14.15 \pm 0.17
28	15.95 \pm 0.29	14.40 \pm 0.14
30	16.87 \pm 0.30	14.60 \pm 0.10
32	18.45 \pm 0.35	14.72 \pm 0.09
34	20.45 \pm 0.27	14.97 \pm 0.11

Plant age (Weeks)	Vernalised conditions Leaf number (Mean \pm S.E)	Non-Vernalised conditions Leaf number (Mean \pm S.E)
20	10.50 \pm 0.64	11.0 \pm 0.70
22	13.50 \pm 0.64	12.25 \pm 0.62
24	16.75 \pm 0.47	13.0 \pm 0.70
26	19.25 \pm 0.47	14.50 \pm 0.5
28	22.0 \pm 0.40	15.75 \pm 0.47
30	24.75 \pm 0.47	16.50 \pm 0.29
32	25.75 \pm 0.47	18.50 \pm 0.64
34	26.0 \pm 0.40	19.50 \pm 0.64

EXPERIMENT 2: EFFECT OF SHADING ON THE GROWTH OF *P. BRACTEATUM*

Shading of plants during the winter months resulted in reduced growth rate. Increasing levels of shading resulted in reduced rate of leaf initiation (Figure 18), shorter leaf length (Figure 19) and plant height (Figure 20). Plants appeared healthy under all shading treatments and symptoms such as formation of necrotic lesions on leaves and high rates leaf senescence that preceded the plant dormancy response noted in previous experiments were not observed.

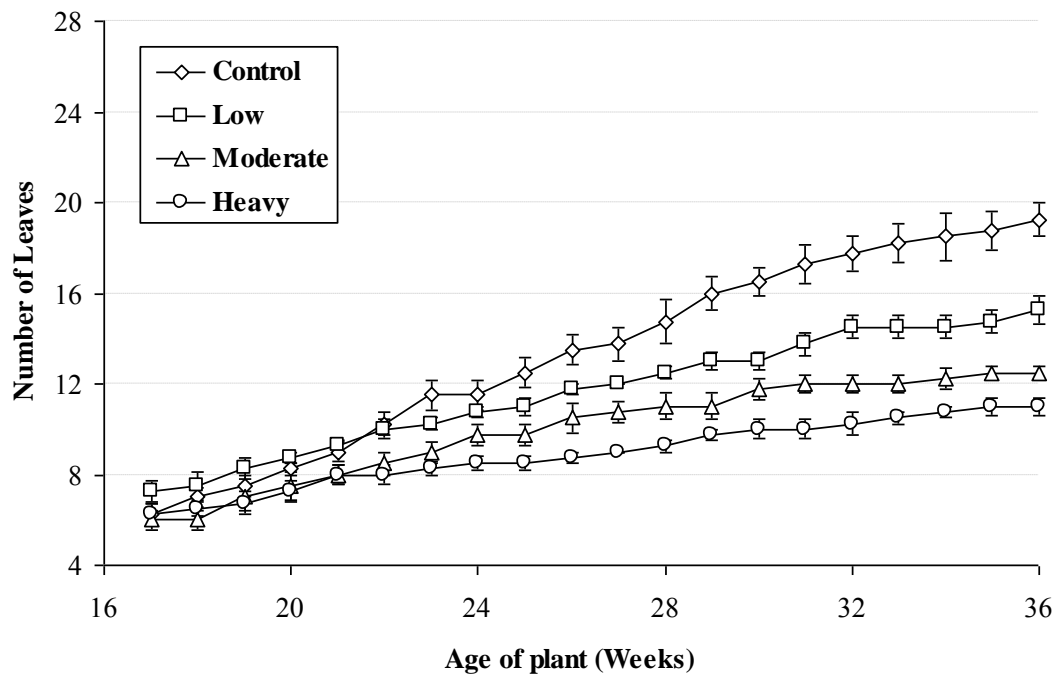


Figure 18: The effect of shade treatments on the number of leaves produced. Points represent the mean leaf numbers of four replicates. Bars represent SEM (n=4).

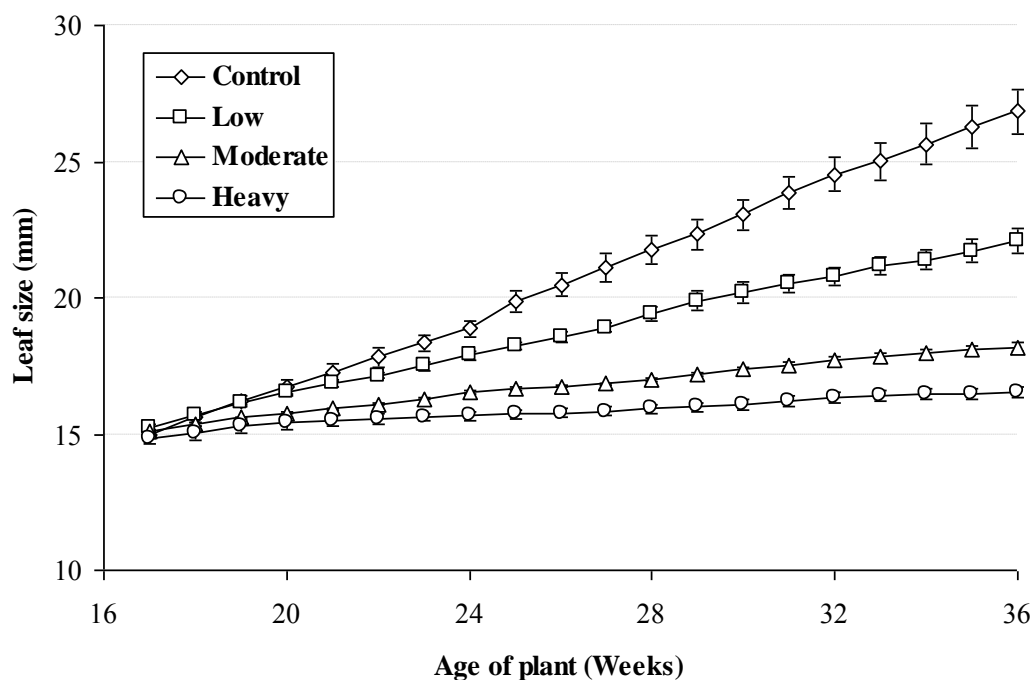


Figure 19: The effect of shade treatments on the leaf size. Points represent the mean time to increase in size of the leaves of four replicates. Bars represent SEM (n=4).

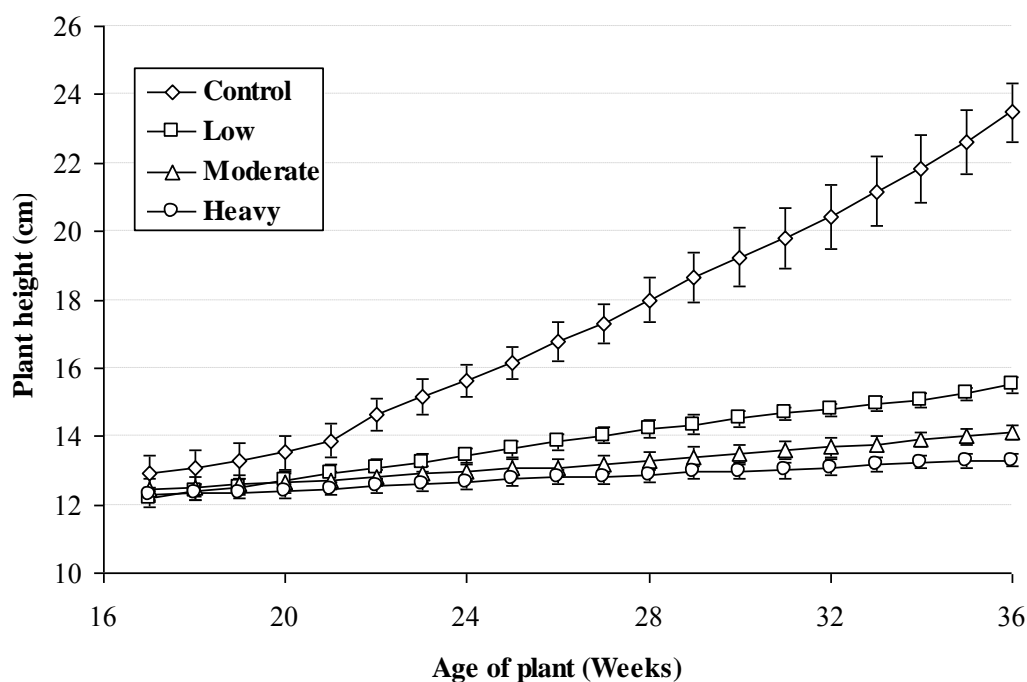


Figure 20: Effect of shade treatments on the plant height. Points represent the mean of four replicates. Bars represent SEM (n=4).

EXPERIMENT 3: EFFECT OF SHADING AND TEMPERATURE ON THE GROWTH OF *P. BRACTEATUM* DURING SUMMER

Plant growth rate under glasshouse conditions was observed to be lower than that under lower temperature conditions outside the glasshouse. All plants grown without shading in the glasshouse displayed leaf necrosis symptoms within 4 weeks of commencement of the experiment and were fully dormant, with no remaining fully expanded green leaves, within 10 weeks of the commencement of the experiment. Plants grown under shade in the glasshouse displayed similar symptoms to the unshaded plants but only four of the fifteen shaded plants became dormant by the end of the experiment. None of the plants placed outside the glasshouse became dormant.

The rate of increase in leaf number was higher in plants growing outside the glasshouse than in plants grown under shading in the glasshouse (Figure 21). Mean leaf number per plant was, however, lowered in the glasshouse population by the loss of leaves in the four plants that became dormant, suggesting that the rate of leaf production may have been more similar in the two environments that the graphical representation suggests. Plant height varied between the two environments, with a more erect leaf habit and longer leaf length noted under outside conditions compared to shaded, glasshouse conditions (Figure 22).

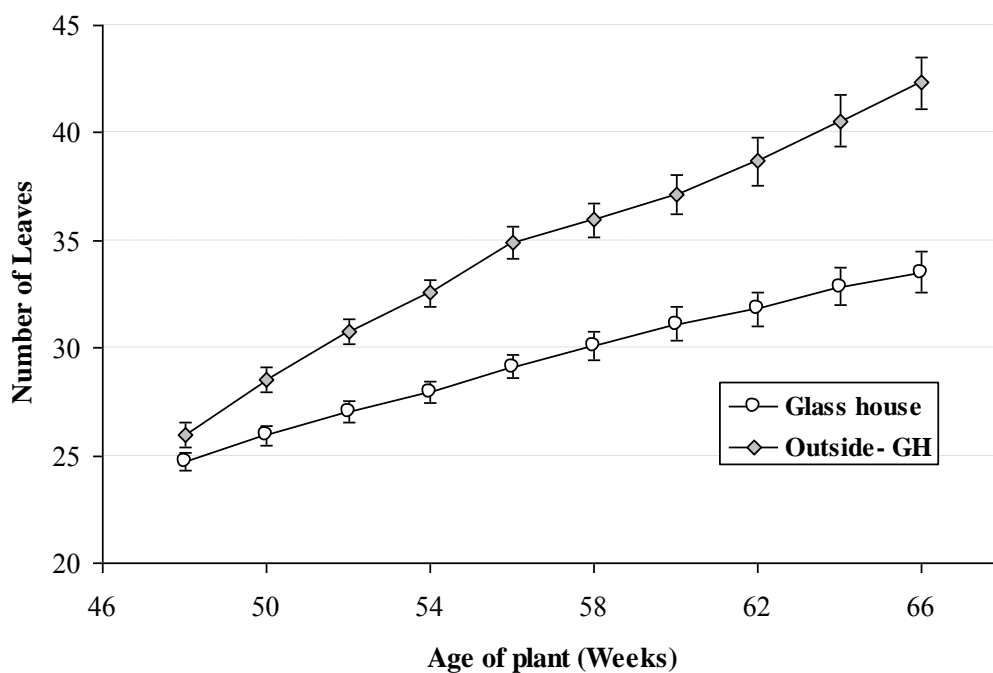


Figure 21: Effect of shading on the number of leaves produced. Points represent the mean leaf numbers of fifteen replicates. Bars represent SEM (n=15).

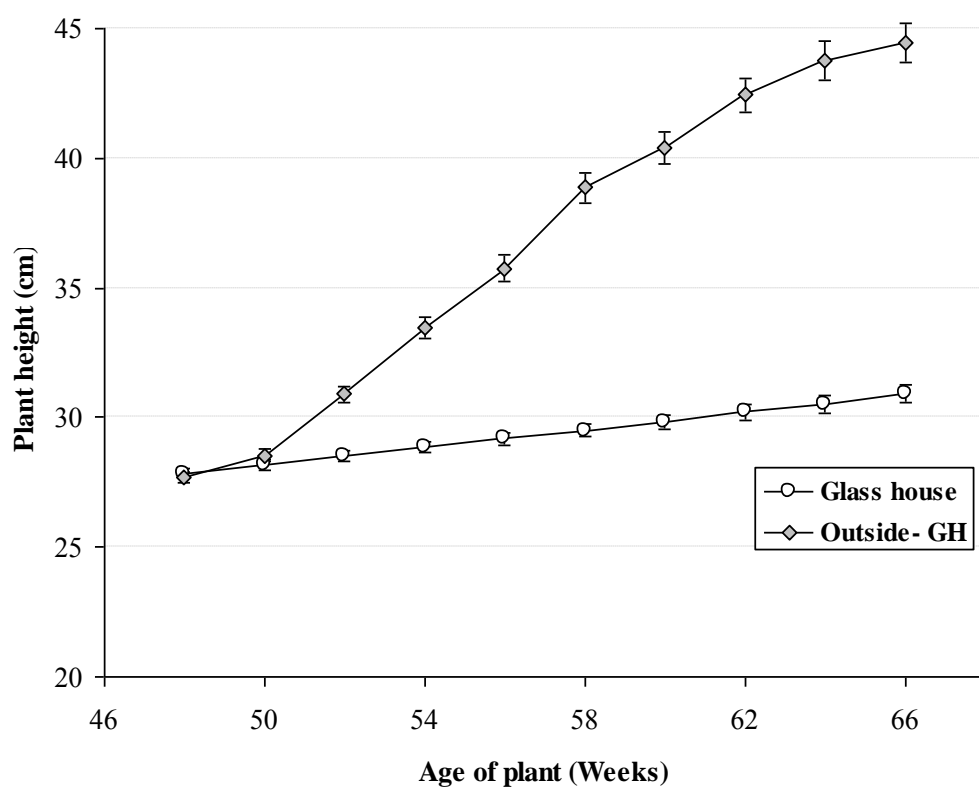


Figure 22: Effect of shading on plant height. Points represent the mean of fifteen replicates. Bars represent SEM (n=15).

EXPERIMENT 4: EFFECT OF GIBBERELIC ACID ON FLOWERING OF *P. BRACTEATUM*

The percentage of plants flowering varied between treatments from 40% of paclobutrazol treated plants and 60% of control plants to 100% of gibberellic acid treated plants (Table 10). Gibberellic acid treatment also induced more rapid and more even flowering, with mean time to visible flower bud of 29.6 days (± 1.03) and mean time to anthesis of 44.7 days (± 0.97). In comparison, mean times to visible bud and anthesis in control and paclobutrazol treated plants that flowered were 31.0 (± 0.80) and 44.33 (± 1.08), and 30.75 (± 0.87) and 48.75 (± 1.35), respectively.

Table 10: Date of first and second application of GA and anti- GA treatments was on 2nd November and 12th November (2009). DVB is number of days to visible bud appearance after first application. DAF is the number of days to flowering after GA and anti- GA application. dnf = did not flower.

Individual plants	GA3		Paclobutrazol		Control	
	DVB	DAF	DVB	DAF	DVB	DAF
1	25	39	dnf	dnf	29	43
2	35	48	dnf	dnf	dnf	dnf
3	31	46	34	55	32	42
4	27	44	dnf	dnf	32	43
5	26	43	29	46	dnf	dnf
6	29	44	dnf	dnf	28	45
7	34	48	dnf	dnf	dnf	dnf
8	28	41	28	46	35	51
9	30	48	dnf	dnf	dnf	dnf
10	31	46	32	48	30	42
Mean (±S.E)	29.6(±1.03)	44.7(±0.97)	30.75(±0.87)	48.75(±1.35)	31.0(±0.80)	44.33(±1.08)

Plants treated with GA₃ had higher numbers of fully expanded leaves and greater plant height than those treated with paclobutrazol or left untreated (Figure 23 and Figure 24). Paclobutrazol treated plants produced shorter, thicker leaves that were darker green in colour compared to control and gibberellic acid treated plants (Plate 4).

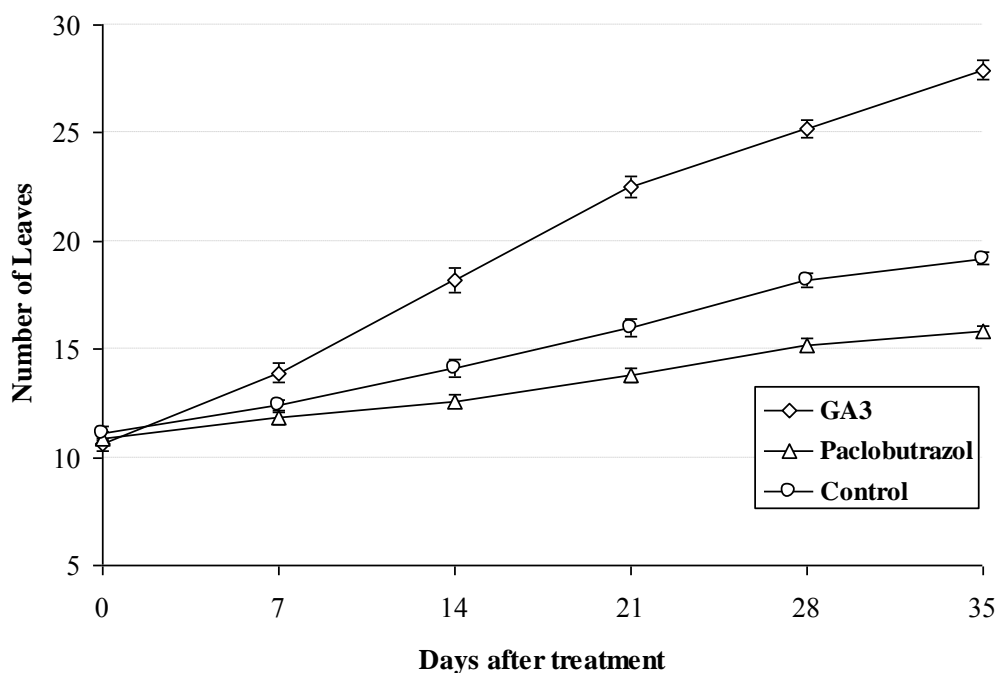


Figure 23: The effect of application of gibberellic acid (GA₃) and paclobutrazol on the number of leaves present on plants. Points represent the mean leaf numbers of ten replicates. Bars represent SEM (n=10).

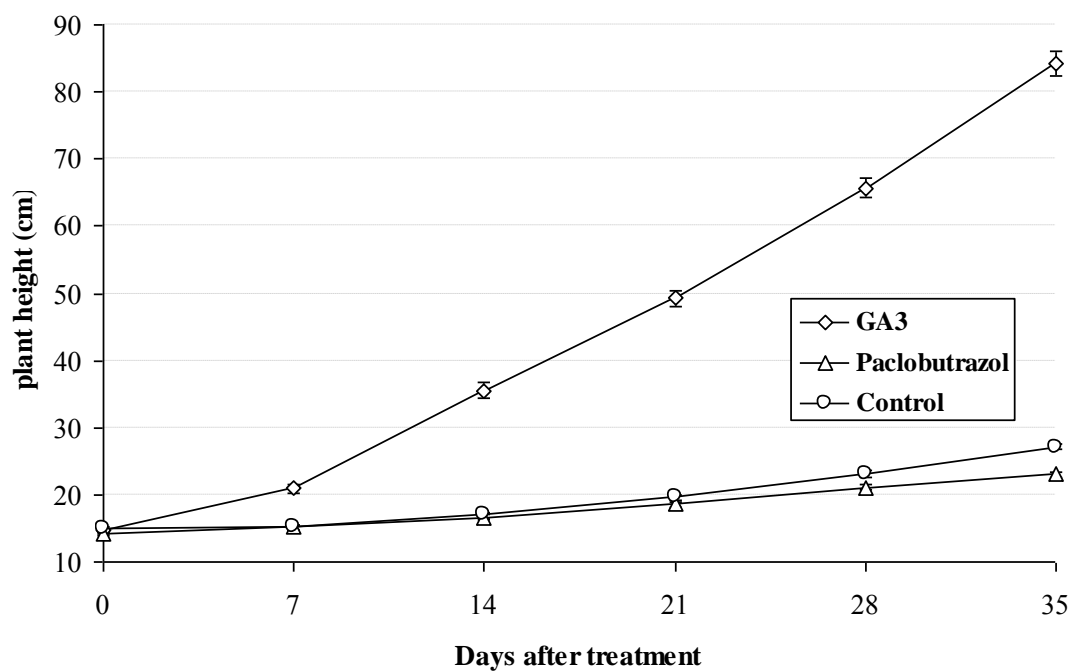


Figure 24: The effect of application of gibberellic acid (GA₃) and paclobutrazol on plant height. Points represent the mean of ten replicates. Bars represent SEM (n=10).

Plate 4: Plants sprayed with GA₃, control and Paclobutrazol after first dose of application.



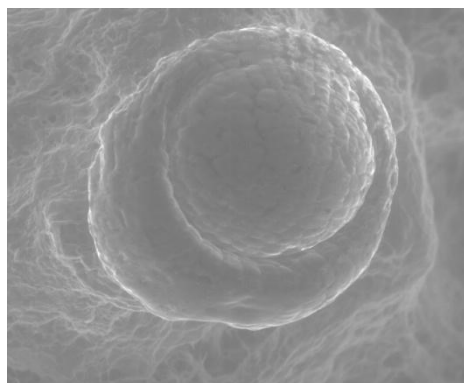
EXPERIMENT 5: SHOOT APICAL MERISTEM MORPHOLOGICAL CHANGES DURING FLOWERING

A series of meristem developmental stages were identified that could be used to describe the early morphological changes associated with the flowering transition and early flower development of *P. bracteatum* (Table 11).

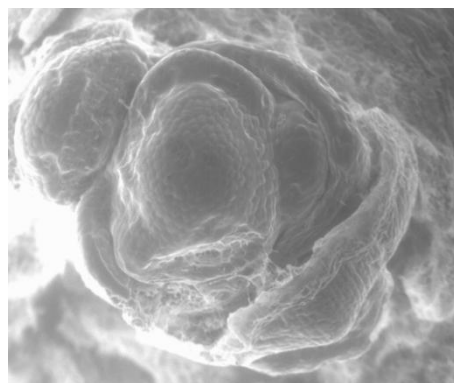
Table 11: Stages of development of apical meristem and developing floral primordia of *P. bracteatum*.

Stage	Description
1	Vegetative meristem with leaf primordia overlapping the meristem.
2	Two initiated bracts are visible. At this stage no floral organs are found within the gynoecium.
3	Secondary floral primordia observed. Four new meristem regions are initiated.
4	Meristematic region of the floral primordia elongates along the vertical axis and apex rises up above half of meristem furthest away from apex.
5	Raised meristem divides into domes (Initiation of anthers). Flowering primordia with reproductive organs. Depression observed in middle of floral primordia. Clearly visible dome and perianth.
6	Half of meristem closest to apex is raised above other half furthest away from apex. Anther and stamen filaments although not visible in the stage have also developed.

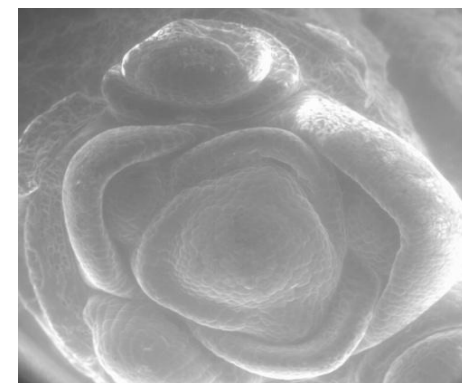
The following images provide a visual description of the meristem development stages.



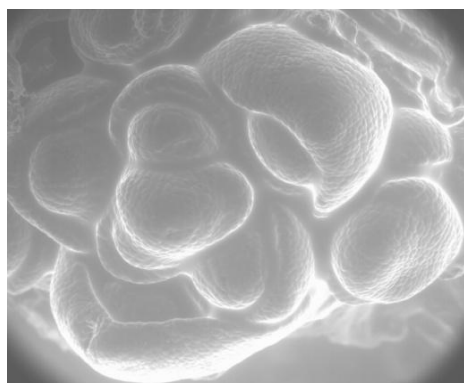
04/May/2010 HV Spot HFW Mag WD Det Pressure Temp —500.0µm—
15:37:22 20.0 kV 4.0 2.70 mm 100x 9.8 mm LFD 0.98 Torr 1.0 °C

STAGE I

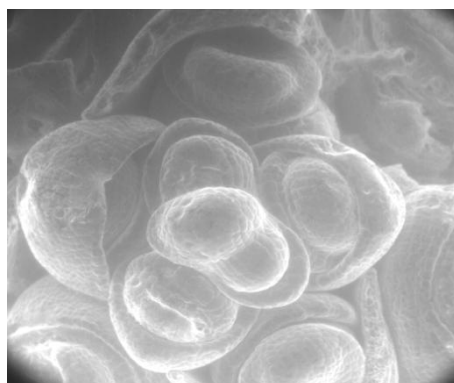
12/May/2010 HV Spot HFW Mag WD Det Sig —2.0mm—
16:26:52 20.0 kV 4.0 10.91 mm 25x 38.7 mm LFD SE

STAGE II

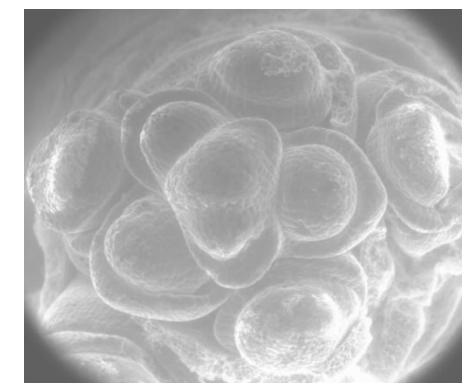
12/May/2010 HV Spot HFW Mag WD Det Sig —2.0mm—
16:06:10 20.0 kV 4.0 9.52 mm 26x 30.1 mm LFD SE

STAGE III

31/May/2010 HV Spot HFW Mag WD Det Temp Pressure Sig —200.0µm—
16:05:03 20.0 kV 4.0 1.82 mm 149x 30.8 mm LFD 1.0 °C 1.00 Torr SE

STAGE IV

31/May/2010 HV Spot HFW Mag WD Det Temp Pressure Sig —1.0mm—
15:56:26 20.0 kV 4.0 12.54 mm 22x 40.3 mm LFD 1.0 °C 1.00 Torr SE

STAGE V

10/Jun/2010 HV Spot HFW Mag WD Det Temp Pressure —2.0mm—
15:48:34 20.0 kV 4.0 18.03 mm 15x 49.3 mm LFD 1.0 °C 1.00 Torr

STAGE VI

DISCUSSION

The evidence generated from the experiments presented in this chapter, and in chapter 5, suggests that *P. bracteatum* has a requirement for a period of vernalisation to induce flowering. This finding is consistent with the species centre of origin where cold winter conditions are experienced and its documented spring time flowering pattern in its natural habitat (Levy *et al.*, 1986). While experiments designed to determine the effects of night temperature and duration of exposure to vernalising conditions could not be completed due to difficulties in plant culture under glasshouse conditions, it was noted that plants exposed to occasional minimum night temperatures of 7.6°C did not flower while flower buds were visible on plants within 4 weeks of exposure to constant 5°C night temperatures. It may therefore be concluded that less than 4 weeks exposure to vernalising night temperatures can induce flowering. Flower buds were visible on untreated plants in the gibberellic acid application experiment as early as 4 weeks after being moved from glasshouse to shadehouse conditions with a mean minimum night temperature of 10°C, suggesting that adequate vernalisation may occur within a night temperature band of at least 5 to 10°C. As only 60% of untreated plants flowered in the experiment, 10°C may be approaching the upper end of the effective temperature range for vernalisation. The similar timing of flowering after vernalisation in shadehouse conditions under long days in summer and under short days in glasshouse conditions in winter suggests that, unlike the annual opium poppy species *P. somniferum* (Wang *et al.*, 1997b), *P. bracteatum* does not have a daylength sensitive flowering response.

Further evidence for a vernalisation requirement for flowering in *P. bracteatum* can be drawn for the response to gibberellic acid application. When grown under shadehouse conditions that partially inductive, promoting flowering in 60% of untreated plants, application of gibberellic acid induced 100% flowering and increased uniformity of flowering. Application of gibberellic acid has previously been reported to substitute for the vernalisation requirement in many perennial species (Cockshull, 1985). Application of the gibberellic acid synthesis inhibitor paclobutrazol resulted in a reduction in flowering percentage, supporting the conclusion that gibberellic acid was involved in the flower induction process in *P. bracteatum*.

The variability in growth rate in *P. bracteatum*, particularly under glasshouse conditions where leaf necrosis and plant dormancy were observed to occur, may have contributed to differences in response to vernalisation treatments imposed in different experiments. The vernalisation stimulus is known to be perceived by the apex and is not translocated within the plant (Napp-Zinn, 1987), with plant material is only sensitive to cold if it contains actively dividing cells in its apical meristem (Bernier *et al.*, 1981a). The transition to the dormant state in glasshouse grown plants suggests reduced mitotic activity may occur under certain growing conditions, and it is plausible that perception of the vernalisation stimulus may therefore vary between plants depending on the status of the meristem. Such a mechanism may explain the delay in flowering noted in Chapter 5 for plants exposed to vernalising conditions in late spring and early summer, a period that precedes the seasonal transition to dormancy noted in a number of trials. High temperatures appear to promote the transition to the dormant state with lower light levels reducing the scale of the effect.

Identification of the early changes in meristem morphology during the flowering transition, and development of a series of defined developmental stages for subsequent flower development, provides a tool for further studies of flower initiation in *P. bracteatum*. Defining the duration of vernalisation required for flower initiation and determining the effect of high day temperatures on the initiation and development of flowers remain areas where further research is required.

CHAPTER 7

GENERAL DISCUSSION AND RECOMMENDATIONS

The research conducted during this project will contribute significantly to the development of *P. bracteatum* as a new perennial crop in Tasmania, Australia. Previous attempts to introduce *P. bracteatum* as a new crop in Tasmania in the 1980's were unsuccessful, with low first season yield and difficulties in crop establishment noted as barriers to commercial production (Laughlin *et al.*, 1998). Low flowering percentage was previously reported in Israel by Palevitch and Levy (1983). The sensitivity of *P. bracteatum* seed to low water potential during germination was identified in this study as a likely cause of poor crop establishment from seed. In addition, reduced germination percentage and rate of germination were noted at temperatures below 20°C suggesting that sowing in autumn as soil temperature decreases may provide sub-optimal conditions for crop establishment. Autumn sowing is however required to ensure plants complete the juvenile phase prior to or during the winter period and may therefore receive sufficient vernalisation to induce flowering and a high yield in the first production season.

The knowledge generated in this project on the effect of environmental and cultural factors on plant development during the juvenile and flowering stages will aid the future development of this crop, as for example predictions can be made utilizing a better understanding of the factors affecting the vegetative and reproductive stages of *P. bracteatum*. Accurate information on the cardinal temperatures required for seed

germination will also assist the industry to schedule the production of the crop based on seasonal weather records for new production locations.

The optimum temperature and moisture conditions for germination were in narrow ranges, and failure to ensure soil moisture and temperature were optimum for germination may have contributed to poor crop establishment results in earlier trials with the species. In comparison to *P. somniferum*, the annual poppy crop currently grown commercially in Tasmania, the maximum germination rate recorded for the two species ranged from 18° to 30°C for *P. bracteatum* and 13° to 33°C for *P. somniferum* (Bare *et al.*, 1978). Growers should expect that the sowing practices currently used for *P. somniferum* will not always deliver adequate crop establishment rates for *P. bracteatum*. Irrigation management is likely to be particularly important as germination was shown to be sensitive to water potential, and like other similarly sensitive species, water stress during germination may decrease or delay seedling emergence, reduce plant growth rate and over the growing season reduce crop biomass (Garwood, 1979; Huang, 1997). Further field based research on time of sowing and management of irrigation following sowing are recommended, with the cardinal temperature data from this study combined with long term average climate data for potential production locations used to narrow down the planting windows in which field trialling should be conducted. Given the sensitivity of *P. bracteatum* seed to low water potential during germination, the development of hydrothermal models to predict seed-lot performance (Gummerson, 1986) is also recommended.

P. bracteatum seed was found to have no physiological dormancy regardless of the growing conditions of the mother plant. Although thermal induced secondary

dormancy has been reported in *P. bracteatum* at high temperatures (Bare *et al.*, 1978), this response was not confirmed in the present study. Physiological dormancy was not detected in seed stored for up to 3 years, and more importantly was not found in freshly harvested seed from mature capsules. This finding is significant for commercial production of the crop as capsule maturity and seed harvest under Tasmanian conditions will occur in late summer or early autumn, corresponding to the likely initial dates in the optimum crop sowing range. Stored seed could be maintained for a period of nine months at 20°C without any commercially significant loss of viability, and for longer periods with a reduction in seed viability of between 0.1 and 0.3% per week or around 20% loss in germination percentage over a 2 year timeframe. These results demonstrate that adequate maintenance of seed quality for commercial plantings will require specialised storage treatments for long term storage. Further research is recommended to identify optimum storage conditions, with low temperature and low seed moisture likely to be the two most effective means of maintaining seed quality in storage (Bonner, 2003).

Germination percentage and germination rate were found to be influenced by both seed size and seed weight, a finding consistent with studies of many other plant species (Gross, 1984). Given that *P. bracteatum* crop establishment in Tasmania will occur in autumn, when soil temperatures are dropping, the capacity to grade seed-lots and select the higher quality fractions to use in situations where soil temperatures are outside the optimum range for germination will provide a valuable crop establishment management tool. Further expansion of the poppy industry to regions in Tasmania where conditions are less favourable for field establishment may require future research on seed treatments to assist in obtaining target densities and uniform

emergence. Seed priming techniques have been used in other crops enhance germination rate and percentage in order to improve crop establishment under sub-optimal conditions (Ellis and Roberts, 1981) and development of priming techniques for *P. bracteatum* is recommended to provide the industry with greater flexibility in management of crop establishment across a broader range of production locations and planting environments.

Data from the project filled a deficit in the literature on the effects of timing of harvest of *P. bracteatum* on seed quality characteristics including rate and uniformity of germination, germination at sub optimal temperatures and seedling quality. Maturity in *P. bracteatum* seed was attained at 70 days after flowering, a result in agreement with other time of harvest studies where later harvests provide highest seed quality (Borthwick, 1931; Demir *et al.*, 2008; Spurr, 2003). While indicators of maturity were not developed, based on the data collected it is suggested that seed harvest not be undertaken until after fully opening of pores on the capsules occurs. Delaying the commercial harvest of *P. bracteatum* seed crops to this point may result in seed loss through the open pores, but is recommended to maximise germination percentage of the seeds.

In other species, indicators of seed maturity such as seed moisture content (Hill and Watkin, 1975; Steiner and Akintobi, 1986) and size of the seed (Ellis *et al.*, 1993) are used to determine the optimum time to high quality harvest seed. Development of such indicators may be necessary in *P. bracteatum* if seed crop production is undertaken under a range of different production environments. In addition, studies have demonstrated that the quality of a seed-lot can be affected by agronomic factors

during seed development on the maternal parent (Ghassemi-Golezani, 1992) as well as harvesting techniques (Oplinger *et al.*, 1989), post-harvest handling (Elias and Copeland, 2001) and storage conditions and duration (Adebisi and Ajala, 2007; Adebisi and Ojo, 2001; McDonald, 2000; TeKrony *et al.*, 1980; Tesnier *et al.*, 2002), and each of these factors provide scope for further research in *P. bracteatum*.

While the development of management strategies to ensure uniform emergence and establishment of a crop stand at the target density is essential in commercial crop production, the capacity to manage a perennial crop such as *P. bracteatum* to ensure a high yield as soon as possible after establishment is critical to the economic viability of the crop (Scheinost *et al.*, 2001). *P. bracteatum* was demonstrated to have an extended juvenile phase of development, lasting approximately 5 months under glasshouse conditions and longer under shadehouse conditions. Phase transition from juvenile to mature phase is a significant stage in the development of many perennial crop plants (Jones, 1999). The duration of this phase in *P. bracteatum* exceeds that of other herbaceous perennials such as *Tanacetum cinerariaefolium* L. (Brown, 1992) and *Heuchera* species (Bressingham Hybrids) (Fausey, 2005). The extended duration of juvenility, combined with a requirement for at least 3 weeks of vernalisation, effectively restricts the crop establishment window to late summer or autumn if high rates of flowering and therefore capsule yield are to be obtained in the same year of crop establishment.

As the duration of the juvenile phase was shown to vary with growing conditions, identification of characteristic changes occurring at the phase transition is required to assess the duration of the juvenile phase. The end of juvenility was defined by the

initiation of a critical number of leaves, with 17 mature, fully expanded leaves concluded to be a useful indicator of the phase change. Leaf number has previously been recommended as an indicator of phase change in other species (Adams *et al.*, 1998; Bradley *et al.*, 1997; Sadik, 1967; Singer and McDaniel, 1986). Flowering *P. bracteatum* plants contain both terminal flower stems and lateral vegetative rosette shoots, with leaf number continuing to increase during flowering through growth of the lateral shoots. The relative growth rates of primary and lateral shoots may vary under differing growing conditions, so further studies under a wider range of conditions, and utilizing a range of germplasm, are recommended to confirm the applicability of leaf number as an indicator of the phase change in *P. bracteatum*.

Higher plants have developed the ability to integrate environmental stimuli and internal signals to control the transition to flowering in natural environments (Ainsworth, 2006; Bernier *et al.*, 1981b). The period of juvenile phase and an obligate requirement for low temperature are integral factors for the reproductive phase transition in *P. bracteatum*. It is likely that the vernalisation requirement is an evolutionary adaptation allowing *P. bracteatum* to flower when conditions are optimal, with the transition from winter to spring in the temperate climate of its origin triggering flowering in the summer period. The vernalisation requirement was shown to be approximately 3 weeks at a 5°C night temperature, with a similar duration where temperature reached a minimum of between 8° and 12°C for part of the night period failing to induce flowering. Further characterisation of the vernalisation requirement across a range of temperatures and duration is required if modelling of the flowering response under marginally inductive conditions is required for selection of appropriate production locations. Factors other than

vernalisation were implicated in the flower initiation and/or development processes, with an increase in the time taken from imposition of vernalisation to anthesis with increasing plant age noted in the study. Exposure to high day time temperatures during vernalisation has been shown to delay or prevent flowering in other species (Schwabe, 1955) and has been referred to as de-vernalisation (Bernier *et al.*, 1981a). It is possible that this response also occurs in *P. bracteatum*, and may have implication for field production of the crop in warmer climatic zones. The documentation in this project of a series of descriptions of meristem development stages during early flowering provides a tool for further study of the possible de-vernalisation phenomena. The observed dormancy phase in *P. bracteatum*, which can cause major problems when conducting plant development studies under glasshouse conditions, may also complicate the flowering response in this species. Characterisation of the dormancy process under both glasshouse and field conditions is recommended as a basis for further examination of the interactions between flower initiation and development and the changes in plant growth rate that precede dormancy.

In addition to a lack of knowledge of the processes triggering dormancy, a number of other issues associated with the cultivation of this species in the glasshouse became apparent during the project. While further refinements in cultural practices to ensure healthy growth of plants under glasshouse conditions are needed, biological control agents are recommended for managing white fly and mites, with chemical treatments used under conditions favouring rapid development of mite populations. Plants were also found to perform best when grown in large 16L planter bags and a capillary matting irrigation, with calcium nitrate foliar applications recommended where leaf

chlorosis symptoms are observed. The evidence of improved plant vigour associated with the use of larger pots and maintenance of soil moisture through capillary matting irrigation, combined with the observations of photo-damage and rapid onset of dormancy under high temperature and high light intensity conditions, suggests that avoiding water stress and/or photostress conditions may reduce the risk of dormancy. This may have implications for field production of *P. bracteatum* and suggests that irrigation management may be critical for the crop not only during crop establishment but also during the summer period when flower and capsule development is likely to be occurring. The routine use of shading to avoid photodamage is not recommended as a decrease in the photosynthetic capacity of plants reduced growth rate and therefore yield potential.

In summary the research conducted during this project indicated that *P. bracteatum* can be successfully grown across Tasmania. The crop has significant potential as an alternative source of opiate alkaloid and offers the additional advantage of being a perennial crop. The data generated from studies of aspects of the biology of the species does not replace the need for field trials to establish crop production recommendations, but provides valuable data to narrow the focus required in field studies. Knowledge of the germination, juvenility and flowering characteristics of the species may be used, in combination with improved cultivars and cultural practices, to develop production strategies that reduce the risk of poor crop establishment and low first year yields, problems that previously led to the conclusion that the crop was not commercially feasible under Tasmanian conditions.

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